USER GUIDE

applied biosystems® by life technologies™

AmpFℓSTR® Identifiler® Direct PCR Amplification Kit

for use with:
200 reaction kit (Part no. 4467831)
1000 reaction kit (Part no. 4408580)

Publication Part Number 4415125 **Rev.** H
Revision Date June 2012

life technologies™

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Contents

About This Guide

IMPORTANT! Before using this product, read and understand the information the "Safety" appendix in this document.

Revision history

Revision	Date	Description
A	May 2009	New document.
B	August 2009	Add Experiments and Results chapter.
C	October 2009	Update screen shots for Panel Manager.
D	September 2010	Change copyright page information.
E	July 2011	Add 200-reaction kit, Bode Buccal DNA Collector™, Prep-n-Go™ Buffer, and 3100-*Avant*, 3130, and 3500/3500xL Genetic Analyzer information.
F	October 2011	Add information for Prep-n-Go™ Buffer to Experiments and Results chapter.
G	March 2012	Change copyright page information.
H	May 2012	Add heat protocol for buccal swab lysate preparation. Add results for additional swab types.

Purpose

The *AmpFℓSTR® Identifiler® Direct PCR Amplification Kit User Guide* provides information about the Life Technologies instruments, chemistries, and software associated with the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit.

1 Overview

Product overview

Purpose

The AmpFℓSTR® Identifiler® Direct PCR Amplification Kit is a short tandem repeat (STR) multiplex assay optimized to allow direct amplification of single-source:

- Blood and buccal samples on treated paper substrates without the need for sample purification.
- Blood and buccal samples collected on untreated paper substrates and treated with Applied Biosystems® Prep-n-Go™ Buffer.
- Buccal samples collected on swab substrates and treated with Applied Biosystems® Prep-n-Go™ Buffer

The Identifiler® Direct Kit amplifies 15 autosomal STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) and the sex-determining marker, Amelogenin, in a single PCR reaction.

Substrate examples

- Treated paper: Copan NUCLEIC-CARD™ system or Whatman FTA® cards
- Untreated paper: Bode Buccal DNA Collector™ or 903 paper
- Swab: Copan FLOQSwabs™

Product description

The Identifiler® Direct Kit contains all the necessary reagents for the amplification of human genomic DNA.

The reagents are designed for use with the following Applied Biosystems® instruments:

- Applied Biosystems® 3100/3100-*Avant* Genetic Analyzer
- Applied Biosystems® 3130/3130*xl* Genetic Analyzer
- Applied Biosystems® 3500/3500xL Genetic Analyzer
- Applied Biosystems® 3730 Genetic Analyzer
- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block
- Veriti® 96-Well Thermal Cycler

About the primers

The Identifiler® Direct Kit employs the same primer sequences as used in the AmpFℓSTR® Identifiler® PCR Amplification Kit. Degenerate primers for the loci D8S1179, vWA, and D16S539 are included in the AmpFℓSTR® Identifiler® Direct Primer Set to address mutations in the primer binding sites. The addition of the degenerate primers allows for the amplification of those alleles in samples containing the mutations without altering the overall performance of the Identifiler® Direct Kit.

Non-nucleotide linkers are used in primer synthesis for the following loci: CSF1PO, D13S317, D16S539, D2S1338, and TPOX. For these primers, non-nucleotide linkers are placed between the primers and the fluorescent dye during oligonucleotide synthesis (Butler 2005, Grossman *et al.*, 1994, and Baron *et al.*, 1996). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate inter-locus spacing. The combination of a five-dye fluorescent system and the inclusion of non-nucleotide linkers allows for simultaneous amplification and efficient separation of the 15 STR loci and Amelogenin during automated DNA fragment analysis.

Loci amplified by the kit

Table 1 shows the loci amplified, their chromosomal locations, and the corresponding fluorescent marker dyes. The AmpFℓSTR® Identifiler® Direct Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder, and the genotype of the AmpFℓSTR® Identifiler® Direct Control DNA 9947A, are also listed in the table.

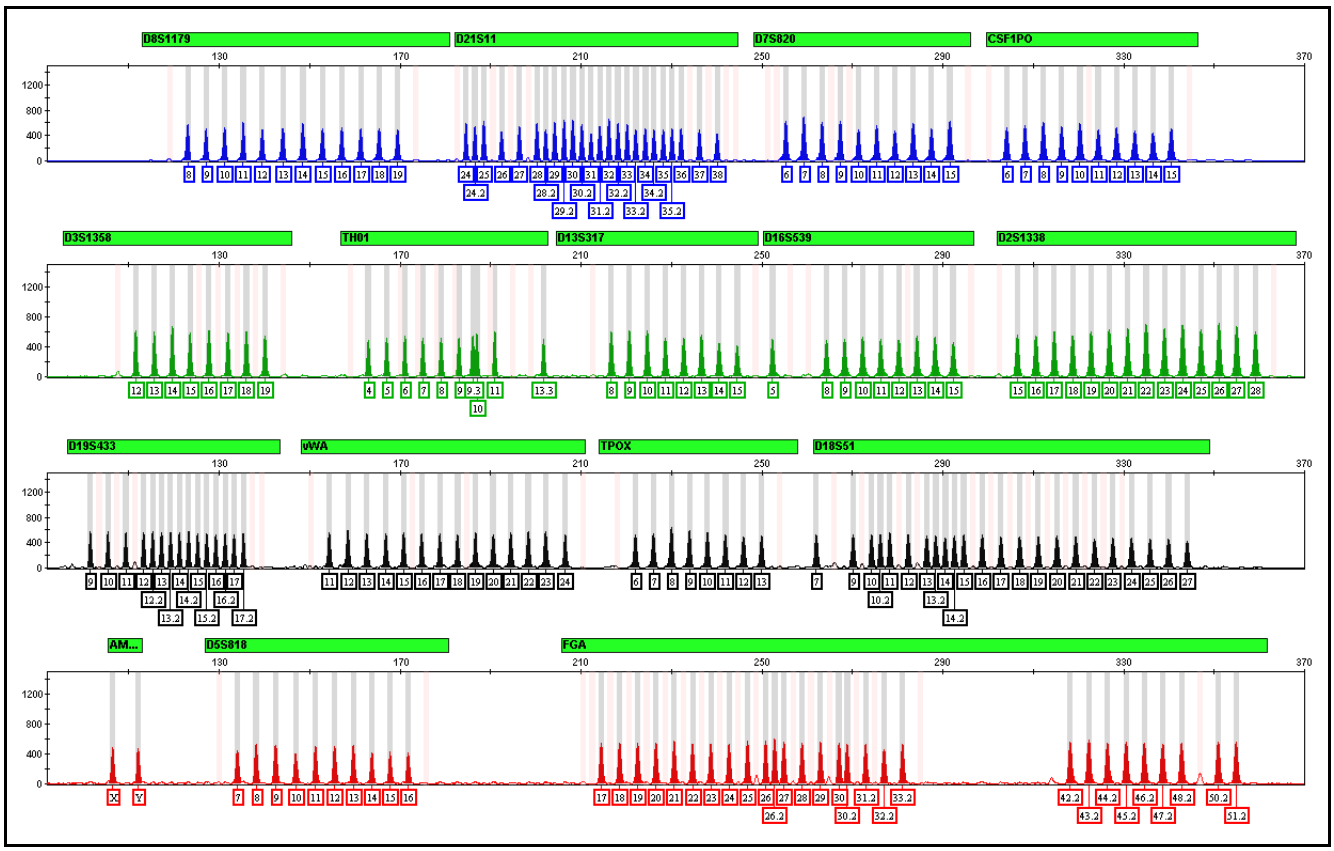
Table 1 AmpFℓSTR® Identifiler® Direct PCR Amplification Kit loci and alleles

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	Control DNA 9947A
D8S1179	8	8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	6-FAM™	13, 13
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38		30, 30
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		10, 11
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		10, 12
D3S1358	3p	12, 13, 14, 15, 16, 17, 18, 19	VIC®	14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		8, 9.3
D13S317	13q22-31	8, 9, 10, 11, 12, 13, 14, 15		11, 11
D16S539	16q24-qter	5, 8, 9, 10, 11, 12,13, 14, 15		11, 12
D2S1338	2q35-37.1	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		19, 23
D19S433	19q12-13.1	9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	NED™	14, 15
vWA	12p12-pter	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		17, 18
TPOX	2p23-2per	6, 7, 8, 9, 10, 11, 12, 13		8, 8
D18S51	18q21.3	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		15, 19
Amelogenin	X: p22.1-22.3; Y: p11.2	X, Y	PET®	X
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11, 11
FGA	4q28	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		23, 24

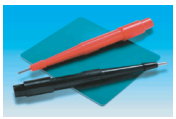
Allelic ladder

Figure 1 shows the allelic ladder for the Identifiler® Direct Kit. See "Allelic ladder requirements" on page 30 for information on ensuring accurate genotyping.

Figure 1 GeneMapper® *ID-X* Software plot of the AmpFℓSTR® Identifiler® Direct Allelic Ladder



Workflow

<table>
<tr><td rowspan="4">Perform PCR</td><td>Obtain samples</td><td>Treated or untreated paper substrates</td><td>Obtain samples</td><td>Swab substrates</td></tr>
<tr><td>Prepare samples</td><td> 
Harris Manual Punch

 
BSD600 Semi-Automated Dried Sample Punch Instrument</td><td>Prepare samples</td><td>Lyse in Prep-n-Go™ Buffer</td></tr>
<tr><td>Prepare reactions</td><td>Untreated paper only: Prep-n-Go™ Buffer

AmpFℓSTR® Identifiler® Direct PCR Amplification Kit</td><td>Prepare reactions</td><td>AmpFℓSTR® Identifiler® Direct PCR Amplification Kit</td></tr>
<tr><td>Perform PCR</td><td colspan="2"> 
GeneAmp® PCR System 9700 Cycler</td><td colspan="2"> 
Veriti® 96-Well Thermal Cycler</td></tr>
<tr><td>Perform electro-phoresis</td><td colspan="4"> 
3100/3100-Avant Genetic Analyzer

 
3130/3130xl Genetic Analyzer

 
3500/3500xL Genetic Analyzer

 
3730 Genetic Analyzer</td></tr>
<tr><td>Analyze data</td><td colspan="4"> 
GeneMapper® ID-X Software

 
GeneMapper® ID Software</td></tr>
</table>

Instrument and software overview

This section provides information about the data collection and analysis software versions required to run the Identifiler® Direct Kit on specific instruments.

Data collection and analysis software

The data collection software provides instructions to firmware running on the instrument and displays instrument status and raw data in real time. As the instrument measures sample fluorescence with its detection system, the data collection software collects the data and stores it. The data collection software stores information about each sample in a sample file (.fsa files for 31xx and 3730 instruments and .hid files for 3500 instruments), which is then analyzed by the analysis software.

Instrument and software compatibility

Instrument	Operating system	Data collection software	Analysis software
3100/3100-*Avant*	Windows® NT	1.1 (3100) 1.0 (3100-*Avant*)	• GeneMapper® *ID* Software v3.2.1 • GeneMapper® *ID-X* Software v1.0.1 or higher
	Windows® 2000	2.0	
3130/3130*xl*†	Windows XP	3.0	
3730†	Windows XP	3.1	
3500/3500xL	• Windows® XP *or* • Windows Vista®	3500 Series Data Collection Software v1.0	GeneMapper® *ID-X* Software v1.2 or higher

† We conducted validation studies for the Identifiler® Direct Kit using these configurations.

About multicomponent analysis

Life Technologies fluorescent multi-color dye technology allows the analysis of multiple loci, including loci that have alleles with overlapping size ranges. Alleles for overlapping loci are distinguished by labeling locus-specific primers with different colored dyes.

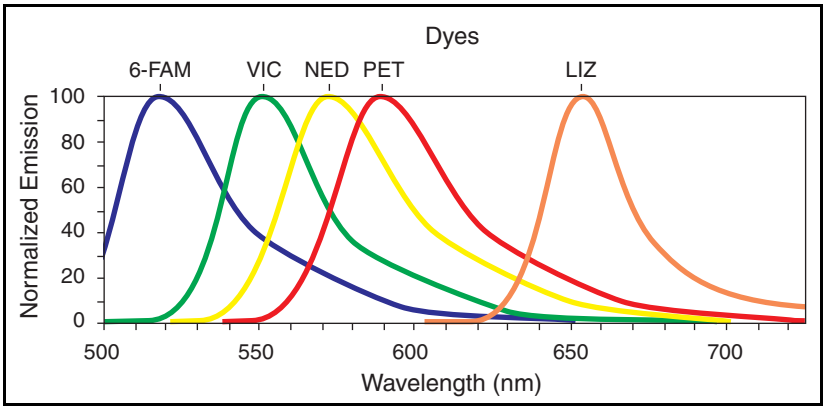
Multicomponent analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes used in the Identifiler® Direct Kit to label samples are 6-FAM™, VIC®, NED™, and PET® dyes. The fifth dye, LIZ®, is used to label the GeneScan™ 500 LIZ® Size Standard or the GeneScan™ 600 LIZ® Size Standard v2.0.

How multicomponent analysis works

Each of these fluorescent dyes emits its maximum fluorescence at a different wavelength. During data collection on Life Technologies instruments, the fluorescence signals are separated by a diffraction grating according to their wavelengths and projected onto a charge-coupled device (CCD) camera in a predictably spaced pattern. The 6-FAM™ dye emits at the shortest wavelength and is displayed as blue, followed by the VIC® dye (green), NED™ dye (yellow), PET® dye (red), and LIZ® dye (orange).

Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes (Figure 2). The goal of multicomponent analysis is to correct for spectral overlap.

Figure 2 Emission spectra of the five dyes used in the Identifiler® Direct Kit



Materials and equipment

Kit contents and storage

The Identifiler® Direct Kit contains sufficient quantities of the following reagents for 200 reactions (Part no. 4467831) or 1000 reactions (Part no. 4408580) at 25 µL/reaction.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Table 2 Kit Contents and Storage

Component	Description	200 reaction	1000 reaction	Storage
AmpFℓSTR® Identifiler® Direct Master Mix	Contains enzyme, salts, dNTPs, carrier protein, and 0.04% sodium azide	2 tubes, 1.25 mL each	1 bottle, 12.5 mL	–15 to –25°C upon receipt, 2 to 8°C after initial use
AmpFℓSTR® Identifiler® Direct Primer Set	Contains forward and reverse primers to amplify human DNA targets.	2 tubes, 1.25 mL each	1 bottle, 12.5 mL	
AmpFℓSTR® Identifiler® Direct Control DNA 9947A	Contains 2 ng/µL human female cell line DNA in 0.04% sodium azide and buffer.† See Table 1 on page 12 for profile.	1 tube, 50.0 µL	1 tube, 50.0 µL	
AmpFℓSTR® Identifiler® Direct Allelic Ladder	Contains amplified alleles. See Table 1 on page 12 for a list of alleles included in the allelic ladder.	1 tube, 50.0 µL	1 tube, 100 µL	

† The Control DNA 9947A is included at a concentration appropriate to its intended use as an amplification control (i.e., to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). The Control DNA 9947A is not designed to be used as a DNA quantitation control and laboratories may expect to see variation from the labelled concentration when quantitating aliquots of the Control DNA 9947A.

Standards for samples

For the Identifiler® Direct Kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **AmpFℓSTR® Identifiler® Direct Control DNA 9947A** – A positive control for evaluating the efficiency of the amplification step and STR genotyping using the AmpFℓSTR® Identifiler® Direct Allelic Ladder.
- **GeneScan™ 500 LIZ® Size Standard or GeneScan™ 600 LIZ® Size Standard v2.0** – Used for obtaining sizing results. These standards, which have been evaluated as internal size standards, yield precise sizing results for Identifiler® Direct Kit PCR products. Order the GeneScan™ 500 LIZ® Size Standard (Part no. 4322682) or the GeneScan™ 600 LIZ® Size Standard v2.0 (Part no. 4408399) separately.
- **AmpFℓSTR® Identifiler® Direct Allelic Ladder** – Developed for accurate characterization of the alleles amplified by the Identifiler® Direct Kit. The Allelic Ladder contains most of the alleles reported for the 15 autosomal loci. Refer to page 12 for a list of the alleles included in the Allelic Ladder.

2 Perform PCR

Optimize PCR cycle number

Before using the Identifiler® Direct Kit for the first time, perform a single initial sensitivity experiment to determine the appropriate cycle number to use during internal validation studies and operational use of the Identifiler® Direct Kit. This experiment accounts for instrument-to-instrument and sample-to-sample variations. If you are processing multiple sample type and substrate combinations (for example, buccal samples on treated paper and buccal samples on swabs), perform separate sensitivity experiments for each sample type and substrate to be used for testing.

The Identifiler® Direct Kit is optimized to amplify unpurified:

- Single-source blood samples on treated paper or untreated paper
- Buccal samples on treated paper, untreated paper, or swabs

When amplifying single-source, unpurified samples using the Identifiler® Direct Kit, you should expect to see greater variation in peak height from sample to sample than is expected with purified samples. Careful optimization of the cycle number will help to minimize this variation.

Select samples and prepare plates

1. Select 26 of each sample+substrate type. Ensure the selected samples represent a "typical" range of samples analyzed in your laboratory.

   IMPORTANT! The number of samples recommended for this study has been chosen to allow you to complete electrophoresis using a single 96-well plate, thus minimizing the impact of run-to-run variation on the results. Examples of PCR and electrophoresis plate layouts are provided on page 109.

2. Prepare the samples and the reactions as described in the protocols later in this chapter. Prepare sufficient PCR reagents to complete amplification of three replicate plates.
3. Create three identical PCR plates (see page 109 for a suggested plate layout).
4. Amplify each plate using a different cycle number to determine the optimum conditions for use in your laboratory. Suggested cycle numbers for different sample type and substrate combinations are listed below:

Sample type	Substrate		
	Treated paper	Untreated paper	Swab
Blood	25, 26, 27 cycles	25, 26, 27 cycles	N/A
Buccal	26, 27, 28 cycles	26, 27, 28 cycles	26, 27, 28 cycles

Note: Our testing has not included blood samples on swab substrates. This sample type is not frequently used for the collection of database or casework reference samples.

Note: To minimize the effect of instrument-to-instrument variation, use the same thermal cycler to amplify all three plates. To maximize result quality, prepare and amplify Plate 1 then repeat for Plates 2 and 3. Do not prepare all three plates simultaneously.

Determine optimum conditions

1. Run the PCR products on the appropriate CE platform using the recommended protocol; see Chapter 3, "Perform Electrophoresis" on page 29.
2. Based on the results of the sensitivity study, select the appropriate PCR cycle number for future experiments.

Our studies indicate the optimum PCR cycle number should generate profiles with the following heterozygote peak heights, with no instances of allelic dropout and minimal occurrence of off-scale allele peaks.

Instrument	Heterozygous peak height
31xx	1000–3000 RFU
3500 Series	3000–12,000 RFU

Treated paper substrates: prepare reactions

Sample prep guidelines

- Do not add water to the wells on the reaction plate before adding the punches. If your laboratory is experiencing static issues with the paper discs, you may prepare and dispense the 25 µL reaction mix into the wells of the reaction plate before adding the punches.
- Make the punch as close as possible to the center of the sample to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.
- For manual punching: Place the tip of a 1.2 mm Harris Micro-Punch on the card, hold the barrel of the Harris Micro-Punch (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch in to the appropriate well on the reaction plate.
- For automated punching: Please refer to the User Guide of your automated or semi-automated disc punch instrument for proper guidance.

Prepare the reactions

1. Add samples to the reaction plate:

Well(s)	Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate...	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control **IMPORTANT!** Do not add a blank disc to the positive control well.	• For 25 cycles	3 µL of Control DNA 9947A
	• For 26 and 27 cycles	2 µL of Control DNA 9947A
	• For 28 cycles	1 µL of Control DNA 9947A
Note: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.		

2. Calculate the volume of each component needed to prepare the reactions, using the table below.

Reaction component	Volume per reaction
Master Mix	12.5 µL
Primer Set	12.5 µL

Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The Identifiler® Direct Kit has been optimized for a 25-µL PCR reaction volume to overcome the PCR inhibition expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of Identifiler® Direct Kit chemistry to generate full STR profiles.

3. Prepare reagents. Thaw the Master Mix and the Primer Set, then vortex for 3 seconds and centrifuge briefly before opening the tubes or bottles.

IMPORTANT! Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8°C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

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4. Pipet the required volumes of components into an appropriately sized polypropylene tube.
5. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
6. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate.
7. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, place a MicroAmp® compression pad (Part no. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti® Thermal Cycler does not require a compression pad.

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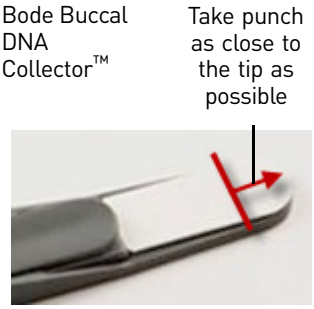
8. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.
9. Amplify the samples in a GeneAmp® PCR System 9700 with the silver or gold-plated silver 96-well block or a Veriti® 96-well Thermal Cycler as described in "Perform PCR" on page 28.

IMPORTANT! The Identifiler® Direct Kit is not validated for use with the GeneAmp PCR System 9700 with the aluminium 96-well block. Use of this thermal cycling platform may adversely affect performance of the Identifiler® Direct Kit.

Untreated paper substrates: prepare reactions

Sample prep guidelines

- Make the punch as close as possible to the center of the sample to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.
- If you are using a Bode Buccal DNA Collector™, make the punch as close as possible to the tip of the DNA collector to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.



- For manual punching: Place the tip of a 1.2 mm Harris Micro-Punch on the card, hold the barrel of the Harris Micro-Punch (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch in to the appropriate well on the reaction plate.
- For automated punching: Please refer to the User Guide of your automated or semi-automated disc punch instrument for proper guidance.

Prepare the reactions

1. Add 2 µL of Prep-n-Go™ Buffer (Part no. 4467079) to the sample and negative control wells in a 96-well plate. Do not add Prep-n-Go™ Buffer to the positive control wells.
2. Add samples to the reaction plate:

Well(s)	Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate...	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control **IMPORTANT!** Do not add a blank disc to the positive control well.	• For 25 cycles	3 µL of Control DNA 9947A
	• For 26 and 27 cycles	2 µL of Control DNA 9947A
	• For 28 cycles	1 µL of Control DNA 9947A
Note: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.		

3. Centrifuge the plate to ensure the punches are immersed in the Prep-n-Go™ Buffer.
4. Calculate the volume of each component needed to prepare the reactions, using the table below.

Reaction component	Volume per reaction
Master Mix	12.5 µL
Primer Set	12.5 µL

Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The Identifiler® Direct Kit has been optimized for a 25-µL PCR reaction volume to overcome the PCR inhibition expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of Identifiler® Direct Kit chemistry to generate full STR profiles.

5. Prepare reagents. Thaw the Master Mix and the Primer Set, then vortex for 3 seconds and centrifuge briefly before opening the tubes or bottles.

IMPORTANT! Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8°C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

6. Pipet the required volumes of components into an appropriately sized polypropylene tube.
7. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
8. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate.
9. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, place a MicroAmp® compression pad (Part no. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti® Thermal Cycler does not require a compression pad.

10. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.
11. Amplify the samples in a GeneAmp® PCR System 9700 with the silver or gold-plated silver 96-well block or a Veriti® 96-well Thermal Cycler as described in "Perform PCR" on page 28.

IMPORTANT! The Identifiler® Direct Kit is not validated for use with the GeneAmp PCR System 9700 with the aluminium 96-well block. Use of this thermal cycling platform may adversely affect performance of the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit.

Swab substrates: prepare reactions

Note: Performance verification and optimization experiments for this protocol were conducted using Copan 4N6 FLOQSwabs®, OmniSwabs, and Puritan swabs air-dried and stored at room temperature for up to three months.

Sample prep guidelines

- Detach buccal swab heads from the swab shaft for lysis.
- Lysis is performed under heated conditions using Prep-n-Go™ Buffer (Part no. 4471406 for buccal swabs) in either of the following formats:
  - 1.5 mL tubes with a heat block (VWR Scientific Select dry heat b lock or similar)
  - 96-well deep-well plate (Part no. 4392904) with an oven and a metal plate adaptor (Robbins Scientific Model 400 Hybridization Incubator or similar, Agilent Benchtop Rack for 200 µl Tubes/V Bottom Plates (metal) Part no. 410094 or similar)

  IMPORTANT! Do not use a plastic plate adaptor.
- For optimum performance, lysis of a whole swab is recommended. To preserve the sample, evaluate lysis of a half swab.

Prepare the sample lysate

1. Preheat the heat block to 90°C or the oven with metal plate adaptor to 99°C.
2. Add 400 µL Prep-n-Go™ Buffer (for buccal swabs, Part. no. 4471406) to 1.5 mL tubes or the appropriate wells of a 96-well deep-well plate (Part no. 4392904).
3. Into each tube or well, put the entire head of each swab. If you are using tubes, cap the tubes. Let the tubes or plate stand for 20 minutes in the preheated heat block or oven to lyse the sample.
4. After 20 minutes, remove the tubes or the deep-well plate from the heat block or oven.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate plate before transferring the lysate.
5. Let the lysate stand at room temperature for at least 15 minutes to cool the lysate (for accurate pipetting).
6. Transfer the sample lysate out of the sample plate into tubes or plates for storage, then discard the deep-well plate containing the swab heads.
7. Proceed to the next section to prepare the reactions or see "Store the sample lysate" on page 27.

Prepare the reactions

1. Calculate the volume of each component needed to prepare the reactions, using the table below.

Reaction component	Volume per reaction
Master Mix	12.5 µL
Primer Set	12.5 µL

Note: Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! This kit has been optimized for a 25-µL PCR reaction volume to overcome the PCR inhibition expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of Identifiler® Direct Kit chemistry to generate full STR profiles.

2. Prepare reagents. Thaw the Master Mix and the Primer Set, then vortex for 3 seconds and centrifuge briefly before opening the tubes or bottles.

IMPORTANT! Thawing is required only during first use of the kit. After first use, reagents are stored at 2 to 8°C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

3. Pipet the required volumes of components into an appropriately sized polypropylene tube.
4. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
5. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate.
6. Add samples to the reaction plate:

Well(s)	Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate...	
Negative control	3 µL of Prep-n-Go™ Buffer	
Test samples	3 µL of lysate	
Positive control	• For 25 cycles	3 µL of Control DNA 9947A
	• For 26 and 27 cycles	2 µL of Control DNA 9947A
	• For 28 cycles	1 µL of Control DNA 9947A

Note: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.

7. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, place a MicroAmp® compression pad (Part no. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti® Thermal Cycler does not require a compression pad.

8. Vortex the reaction mix at medium speed for 3 seconds.
9. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.

10. Amplify the samples in a GeneAmp® PCR System 9700 with the silver or gold-plated silver 96-well block or a Veriti® 96-well Thermal Cycler as described in "Perform PCR" on page 28.

IMPORTANT! The Identifiler® Direct Kit is not validated for use with the GeneAmp PCR System 9700 with the aluminium 96-well block. Use of this thermal cycling platform may adversely affect performance of the Identifiler® Direct Kit.

Store the sample lysate

Cap the sample lysate storage tubes or seal the sample lysate storage plate with MicroAmp® Clear Adhesive Film.

Store the sample lysate as needed:

If you are storing the sample lysate...	Then place at...
<2 weeks	2 to 8°C
>2 weeks	–15 to –25°C

These storage recommendations are preliminary pending the results of ongoing stability studies. The effects of multiple freeze-thaw cycles on the lysate have not been fully evaluated. Therefore, multiple freeze-thaw cycles are not recommended.

Perform PCR

1. Program the thermal cycling conditions.

IMPORTANT! When using the GeneAmp PCR System 9700 with either 96-well silver or gold-plated silver block, select **9600 Emulation Mode**.

Initial incubation step	Optimum cycle number†			Final extension	Final hold
	Denature	Anneal	Extend		
HOLD	**CYCLE**			**HOLD**	**HOLD**
95°C 11 min	94°C 20 sec	59°C 2 min	72°C 1 min	60°C 25 min	4°C ∞

† Determine the optimum cycle number for your laboratory according to the instructions on page 19.

2. Load the plate into the thermal cycler and close the heated cover.
3. Start the run.
4. On completion of the run, store the amplified DNA.

If you are storing the DNA...	Then place at...
<2 weeks	2 to 8°C
>2 weeks	–15 to –25°C

IMPORTANT! Protect the amplified products from light.

3 Perform Electrophoresis

Allelic ladder requirements

To accurately genotype samples, you must run an allelic ladder sample along with the unknown samples.

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder(s)
3100-*Avant* or 3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3100 or 3130*xl*	1 per injection	16 samples	15 samples + 1 allelic ladder
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500xL	1 per injection	24 samples	23 samples + 1 allelic ladder
3730	2 per injection	48 samples	46 samples + 2 allelic ladders

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between both single- and multiple-capillary runs (with larger size variations seen between samples injected in multiple-capillary runs). We recommend the above frequency of allelic ladder injections, which should account for normal variation in run speed. However, during internal validation studies, verify the required allelic ladder injection frequency to ensure accurate genotyping of all samples in your laboratory environment.

It is critical to genotype using an allelic ladder run under the same conditions as the samples, because size values obtained for the same sample can differ between instrument platforms because of different polymer matrices and electrophoretic conditions.

Section 3.1 3100/3100-*Avant* and 3130/3130*xl* instruments

Set up the 3100/3100-*Avant* and 3130/3130*xl* instruments for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 105 lists the required materials not supplied with the Identifiler® Direct Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Electrophoresis software setup and reference documents

The following table lists data collection software and the run modules that can be used to analyze Identifiler® Direct Kit PCR products. For details on the procedures, refer to the documents listed in the table.

Genetic Analyzer	Data Collection Software	Operating System	Run modules and conditions	References
Applied Biosystems® 3100-*Avant*	1.0	Windows® NT	• GeneScan36Avb_DyeSetG5Module Injection condition: 3 kV/5sec • GS600v2.0Analysis.gsp	*3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin* (Part no. 4332345)
Applied Biosystems® 3100	2.0	Windows® 2000	• HIDFragmentAnalysis36_POP4_1 Injection condition: 3kV/10 sec • Dye Set G5	*3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0, Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin* (Part no. 4350218)
	1.1	Windows® NT	• GeneScan36vb_DyeSetG5Module Injection condition: 3kV/10 sec • GS600v2.0Analysis.gsp	*3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin* (Part no. 4332345)
Applied Biosystems® 3130/3130*xl*	3.0†	Windows® XP	• HIDFragmentAnalysis36_POP4_1 Injection conditions: – 3130 = 3 kV/5 sec – 3130*xl* = 3 kV/10 sec • Dye Set G5	*Applied Biosystems 3130/3130xl Genetic Analyzers Using Data Collection Software v3.0, Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin* (Part no. 4363787)

† We conducted validation studies for the Identifiler® Direct Kit using this configuration.

Prepare samples for electrophoresis on the 3100/3100-*Avant* or 3130/3130*xl* instruments

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and size standard needed to prepare the samples:

Reagent	Volume per reaction		Reagent	Volume per reaction
GeneScan™ 500 LIZ® Size Standard	0.3 µL	*OR*	GeneScan™ 600 LIZ® Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	8.7 µL		Hi-Di™ Formamide	8.5 µL

Note: Include additional samples in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp® Optical 96-Well Reaction Plate, add:
   - 9 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 10 µL of Hi-Di™ Formamide.
5. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
6. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Prepare the plate assembly on the autosampler.
9. Start the electrophoresis run.

Section 3.2 3500/3500xL instruments

Set up the 3500/3500xL instruments for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 105 lists the required materials not supplied with the Identifiler® Direct Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Electrophoresis software setup and reference documents

The following table lists data collection software and the run modules that you can use to analyze Identifiler® Direct Kit PCR products. For details on the procedures, refer to the documents listed in the table.

Genetic Analyzer	Data Collection Software	Operating System	Run modules and conditions	References
Applied Biosystems® 3500	3500 Data Collection Software v1.0	Windows® XP *or* Windows Vista®	• HID36_POP4 Injection conditions: 1.2kV/15 sec • Dye Set G5	*Applied Biosystems® 3500/ 3500xL Genetic Analyzer User Guide* (Part no. 4401661) *3500 and 3500xL Genetic Analyzers Quick Reference Card* (Part no. 4401662)
Applied Biosystems® 3500xL			• HID36_POP4 Injection conditions: 1.2kV/24 sec • Dye Set G5	

Prepare samples for electrophoresis on the 3500/3500xL instruments

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and GeneScan™ 600 LIZ® Size Standard v2.0 needed to prepare the samples:

Reagent	Volume per reaction
GeneScan™ 600 LIZ® Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	8.5 µL

Note: Include additional samples in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp® Optical 96-Well Reaction Plate, add:
   - 9 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 10 µL of Hi-Di™ Formamide.
5. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
6. Heat the plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Place the sample tray on the autosampler.
9. Start the electrophoresis run.

Section 3.3 3730 instrument

Set Up the 3730 instrument for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 105 lists the required materials not supplied with the Identifiler® Direct Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use. Keep freeze-thaw cycles to a minimum.

Electrophoresis software setup and reference documents

The following table lists data collection software and the run modules that you can use to analyze Identifiler® Direct Kit PCR products. For details on the procedures, refer to the documents listed in the table.

Operating system	Data collection software	Run module	References
Windows XP	3.0†	• GeneMapper_36_POP7 • Dye Set G5_RCT	Applied Biosystems® *3730 DNA Analyzer Human Identification Validation Report*‡

† We conducted concordance studies for the Identifiler® Direct Kit using this configuration.

‡ Contact your sales or support representative to obtain a copy of the *3730 DNA Analyzer Human Identification Validation Report*.

Prepare samples for electrophoresis on the 3730 instrument

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and size standard needed to prepare the samples:

Reagent	Volume per reaction		Reagent	Volume per reaction
GeneScan™ 500 LIZ® Size Standard	0.3 µL	*OR*	GeneScan™ 600 LIZ® Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	8.7 µL		Hi-Di™ Formamide	8.5 µL

Note: Include additional samples in your calculations to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp® Optical 96-Well Reaction Plate, add:
   - 9 µL of the formamide:size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   Note: For blank wells, add 10 µL of Hi-Di™ Formamide.
5. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
6. Heat the plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Place the sample tray on the autosampler.
9. Start the electrophoresis run.

4 Analyze Data

Section 4.1 GeneMapper® *ID* Software

Overview of GeneMapper® *ID* Software

GeneMapper® *ID* Software is an automated genotyping software for forensic casework, databasing, and paternity data analysis.

After electrophoresis, the Data Collection Software stores information for each sample in an .fsa file. Using GeneMapper® *ID* Software v3.2.1 software, you can then analyze and interpret the data from the .fsa files.

Instruments

Refer to "Instrument and software overview" on page 15 for a list of compatible instruments.

Before you start

When using GeneMapper® *ID* Software v3.2.1 to perform human identification (HID) analysis with AmpFℓSTR® kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Perform the appropriate internal validation studies if you want to use multiple ladder samples in an analysis.

  For multiple ladder samples, the GeneMapper® *ID* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.

When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.

- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples, to ensure proper allele calling.
- Alleles that are not in the AmpFℓSTR® Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, verify the sample result according to your laboratory's protocol.

Set up GeneMapper® *ID* Software for data analysis

File names

The file names shown in this section may differ from the file names you see when you download or import files. If you need help determining the correct files to use, contact your local Life Technologies Human Identification representative, or go to **www.lifetechnologies.com**.

Before using the software for the first time

To analyze sample files (.fsa) using GeneMapper® *ID* Software v3.2.1 for the first time:

- Import panels and bins into the Panel Manager, as explained in "Import panels and bins" on page 38.
- Create an analysis method, as explained in "Create an analysis method" on page 42.
- Create a size standard, as explained in "Create a size standard" on page 47.
- Define custom views of analysis tables.

  Refer to the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Part no. 4335523) for more information.
- Define custom views of plots.

  Refer to the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Part no. 4335523) for more information.

Import panels and bins

To import the Identifiler® Direct Kit panel and bin set from the Life_Technologies web site into the GeneMapper® *ID* Software v3.2.1 database:

1. Download and open the file containing panels and bins:
   a. From the Support menu of **www.lifetechnologies.com**, select **Support ▸ Software Downloads, Patches & Updates ▸** GeneMapper® *ID* Software **v 3.2 ▸ Updates & Patches**, and download the file **Identifiler Direct Analysis Files GMID**.
   b. Unzip the file.

2. Start the GeneMapper® *ID* Software, then log in with the appropriate user name and password.

   IMPORTANT! For logon instructions, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (Part no. 4338775).

3. Select **Tools** ▸ **Panel Manager**.

4. Find, then open the folder containing the panels and bins:

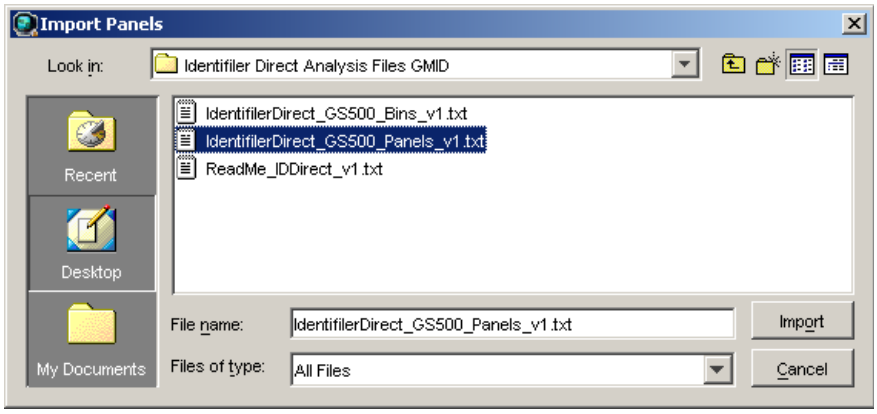
   a. Select **Panel Manager** in the navigation pane.

   b. Select **File** ▸ **Import Panels** to open the Import Panels dialog box.

   c. Navigate to, then open the **Identifiler Direct Analysis Files GMID** folder that you unzipped in step 1 on page 38.

5. Select **IdentifilerDirect_GS500_Panels_v1.txt**, then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager, IdentifilerDirect_GS500_v1. This folder contains the panel and associated markers.

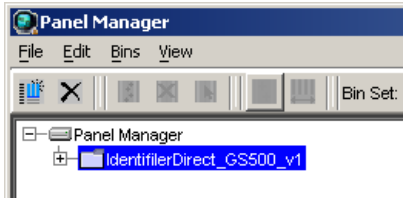


6. Import IdentifilerDirect_GS500_Bins_v1.txt:

   a. Select the **IdentifilerDirect_GS500_v1** folder in the navigation pane.

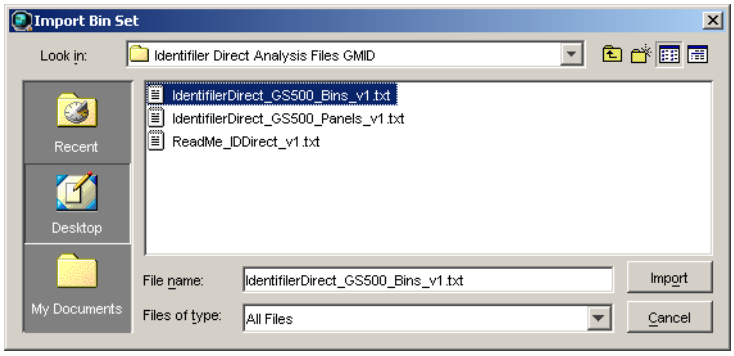
   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

   c. Navigate to, then open the **Identifiler Direct Analysis Files GMID** folder.



d. Select **IdentifilerDirect_GS500_Bins_v1.txt**, then click **Import**.

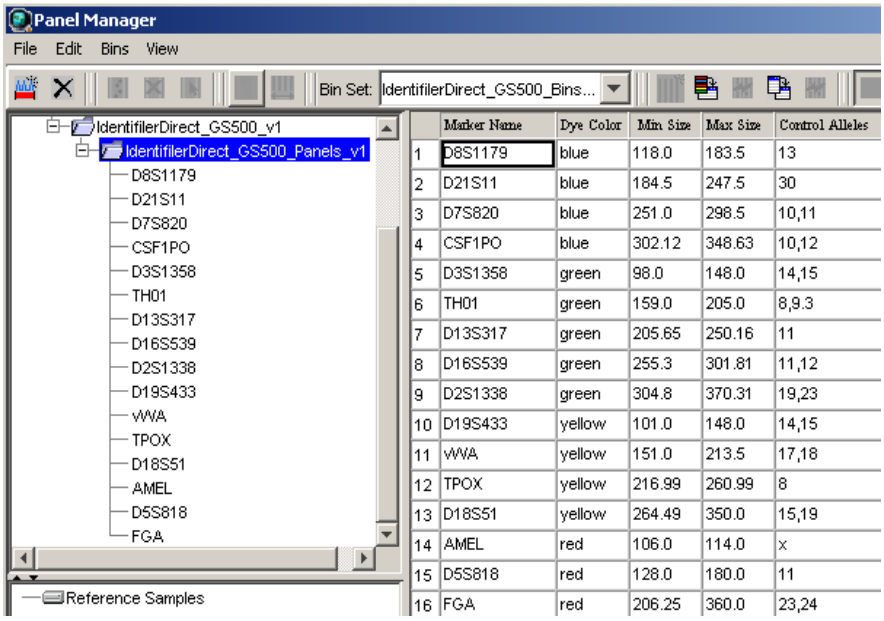
Note: Importing this file associates the bin set with the panels in the IdentifilerDirect_GS500_Panels_v1 folder.



7. View the imported panels in the navigation pane:

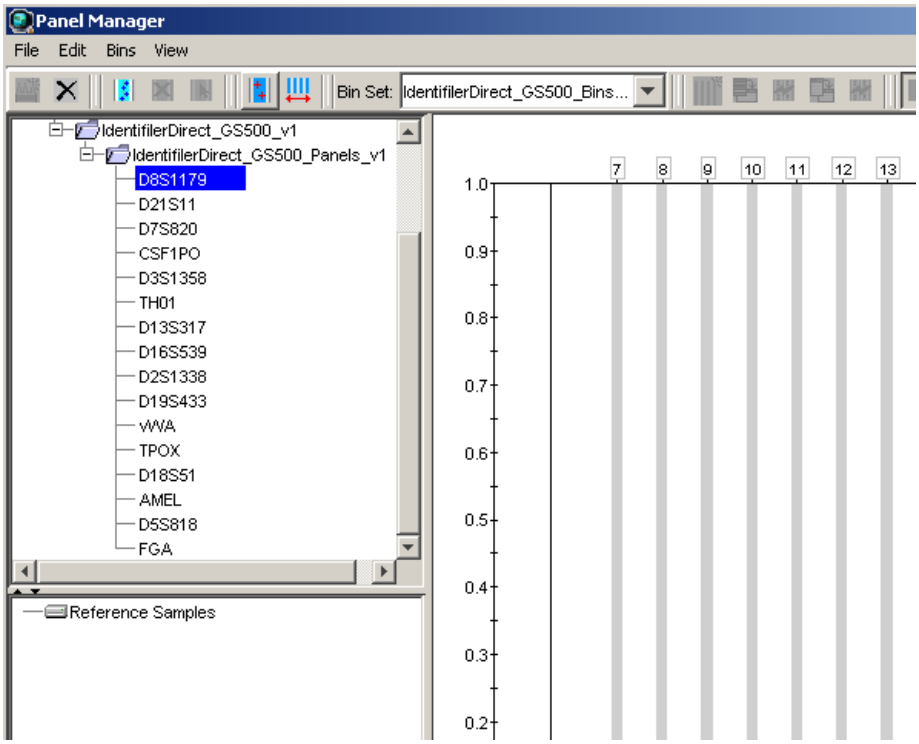
a. Double-click the **IdentifilerDirect_GS500_v1** folder to view the IdentifilerDirect_GS500_Panels_v1 folder.

b. Double-click the **IdentifilerDirect_GS500_Panels_v1** folder to display the panel information in the right pane.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles
1	D8S1179	blue	118.0	183.5	13
2	D21S11	blue	184.5	247.5	30
3	D7S820	blue	251.0	298.5	10,11
4	CSF1PO	blue	302.12	348.63	10,12
5	D3S1358	green	98.0	148.0	14,15
6	TH01	green	159.0	205.0	8,9.3
7	D13S317	green	205.65	250.16	11
8	D16S539	green	255.3	301.81	11,12
9	D2S1338	green	304.8	370.31	19,23
10	D19S433	yellow	101.0	148.0	14,15
11	vWA	yellow	151.0	213.5	17,18
12	TPOX	yellow	216.99	260.99	8
13	D18S51	yellow	264.49	350.0	15,19
14	AMEL	red	106.0	114.0	X
15	D5S818	red	128.0	180.0	11
16	FGA	red	206.25	360.0	23,24

8. Select **D8S1179** to display the Bin view for the marker in the right pane.



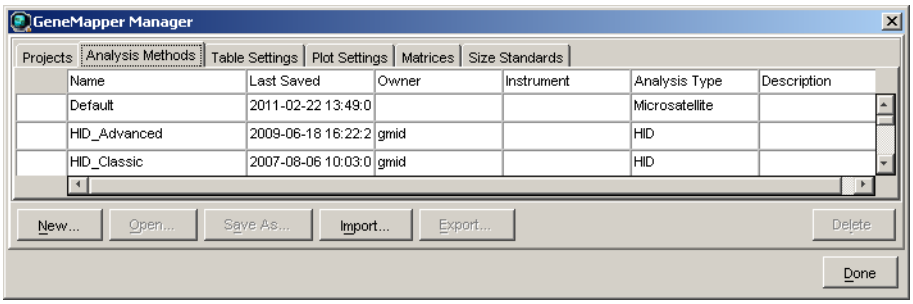
9. Click **Apply**, then **OK** to add the Identifiler® Direct Kit panel and bin set to the GeneMapper® *ID* Software database.

IMPORTANT! If you close the Panel Manager without clicking OK, the panels and bins are not imported into the GeneMapper® *ID* Software database.

Create an analysis method

To create an HID analysis method for the Identifiler® Direct Kit.

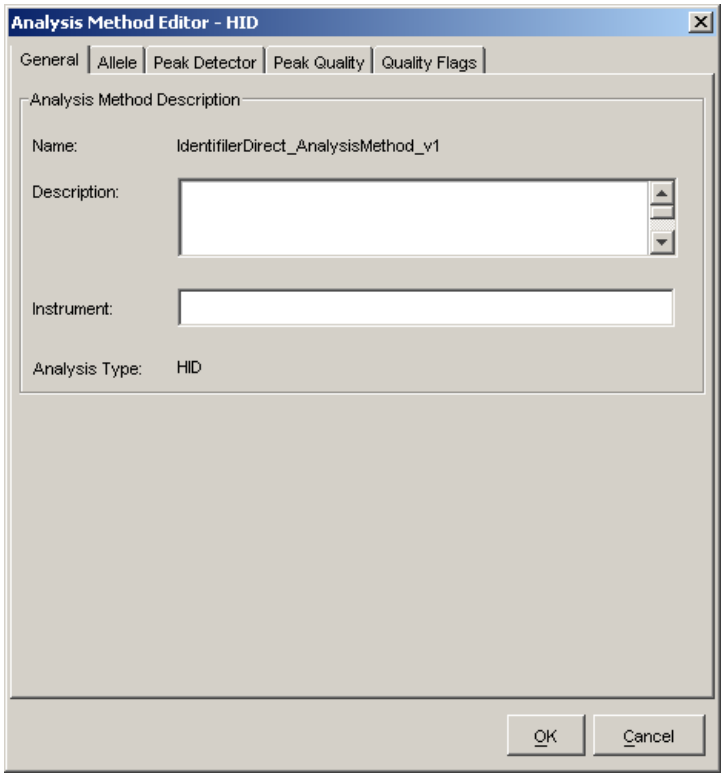
1. Select **Tools ▸ GeneMapper Manager** to open the GeneMapper Manager.



2. Select the **Analysis Methods** tab, then click **New** to open the New Analysis Method dialog box.
3. Select **HID** and click **OK** to open the Analysis Method Editor with the General tab selected.
4. Enter the settings shown in the figures on the following pages.

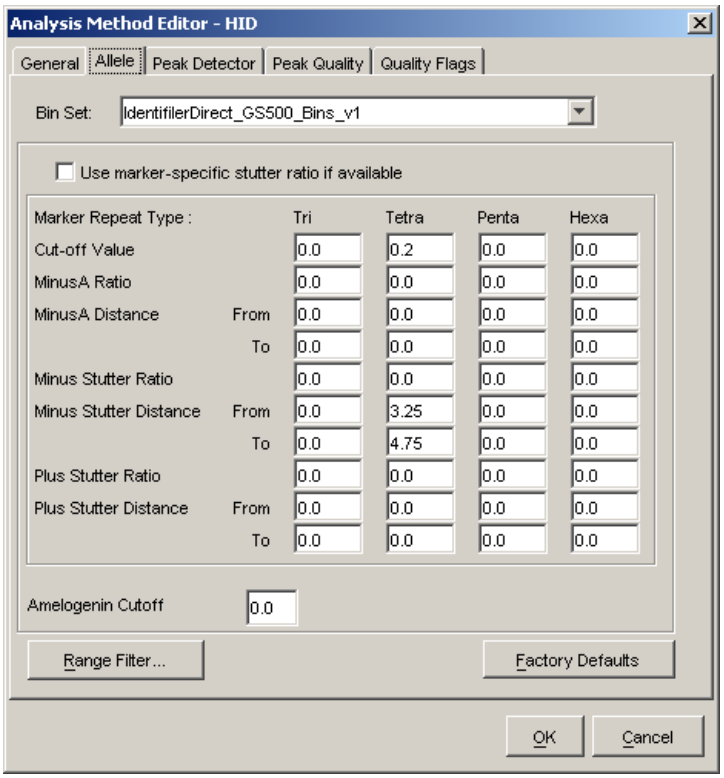
   Note: The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.
5. After you enter settings in all tabs, click **Save**.

General tab settings



In the Name field, either type the name as shown for consistency with files supplied with other AmpFℓSTR® kits, or enter a name of your choosing. The Description and Instrument fields are optional.

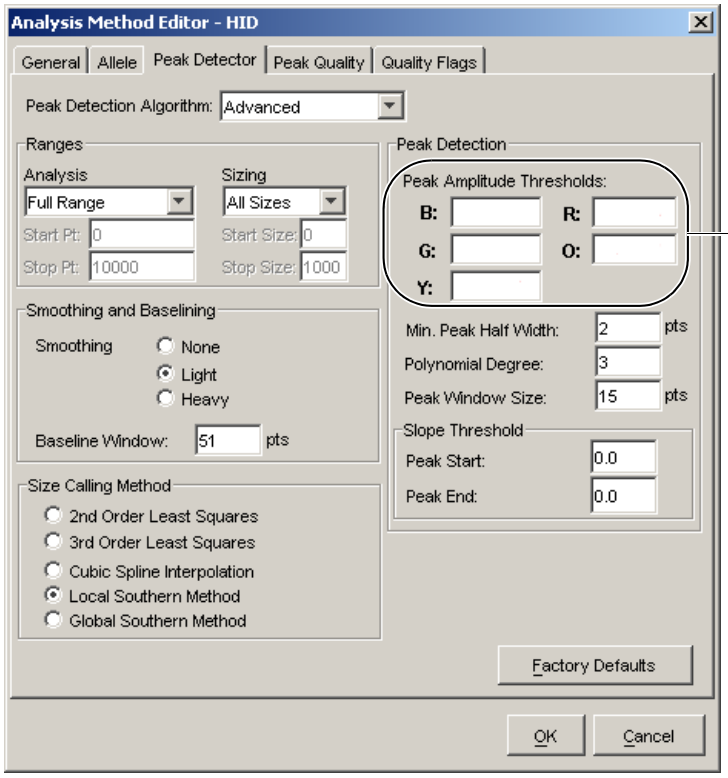
Allele tab settings



- In the Bin Set field, select the **IdentifilerDirect_GS500_Bins_v1** bin set imported previously and configure the stutter distance parameters as shown.
- GeneMapper® *ID* Software v3.2.1 allows you to specify four types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the stutter ratio:
  - To apply the stutter ratios listed in the Allele tab for single-source data, deselect the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

    Note: Applying global stutter ratios may reduce the editing required for single-source sample data.
  - To apply the stutter ratios contained in the IdentifilerDirect_GS500_Panels_v1 file, select the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

Peak Detector tab settings



IMPORTANT! Perform the appropriate internal validation studies to determine the peak amplitude thresholds for interpretation of Identifiler® Direct Kit data.

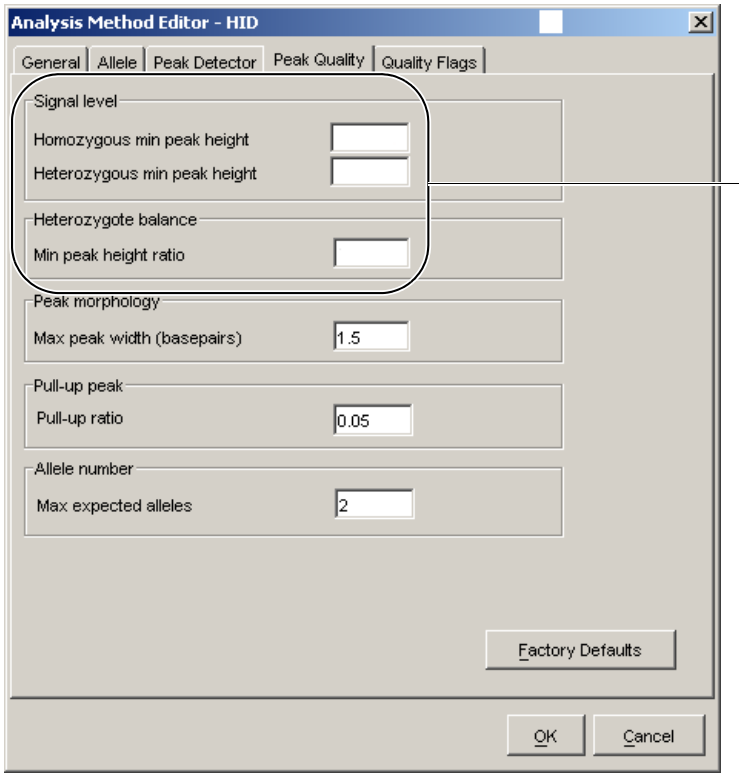
Fields include:

- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper® *ID* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – The Identifiler® Direct Kit has been validated using the Local Southern sizing method. Before using alternative sizing methods, perform the appropriate internal validation studies.

3500/3500xL and 3730 data

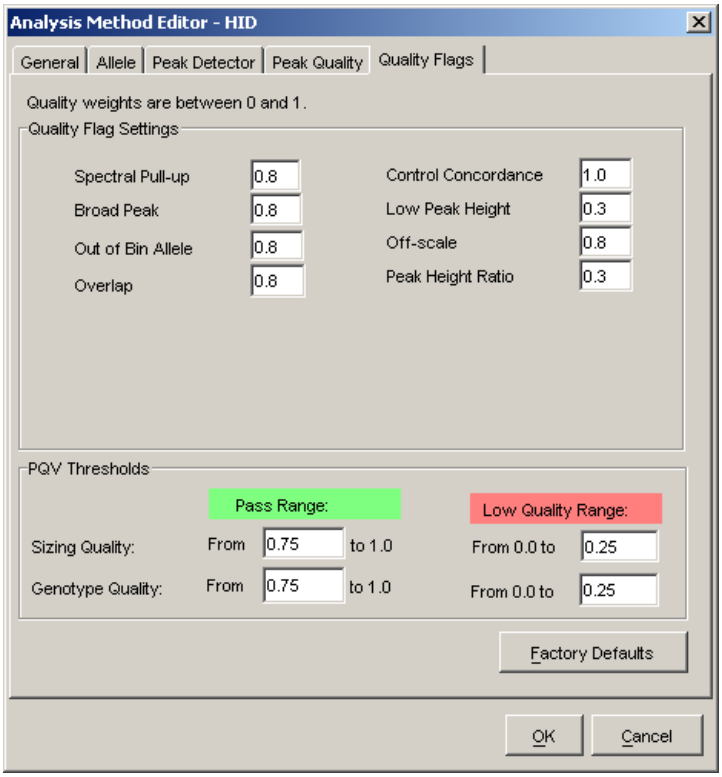
- **3500/3500xL and 3730 data**: Overall peak heights for the data are approximately 3 times higher than peak heights obtained for samples run on the 31xx series instruments. Evaluate validation data carefully to determine the appropriate Peak Amplitude Thresholds for reliable analysis.
- **3730 data only**: Due to differences in the resolution of peaks using POP-7™ polymer versus POP-4® polymer, reduce the Peak Window Size setting in GeneMapper® *ID* Software from 15 pts to 11 pts to obtain accurate genotyping results.
- For more information:
  - Refer to *User Bulletin: Applied Biosystems® 3500/3500xL Genetic Analyzer: Protocols for Analysis of AmpFℓSTR® PCR Amplification Kit PCR Products and Validation Summary* (Part no. 4469192)
  - Contact your sales or support representative to obtain a copy of the *3730 DNA Analyzer Human Identification Validation Report*

Peak Quality tab settings



IMPORTANT! Perform the appropriate internal validation studies to determine the minimum heterozygous and homozygous minimum peak height thresholds and the minimum peak height ratio threshold that allow for reliable interpretation of Identifiler® Direct Kit data.

Quality Flags tab settings



IMPORTANT! The values shown are the software defaults and are the values we used during developmental validation. Perform the appropriate internal validation studies to determine the appropriate values to use in your laboratory.

Create a size standard

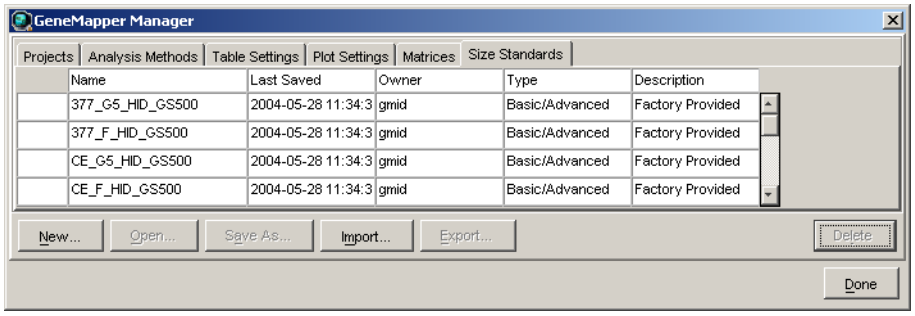
The size standards for the Identifiler® Direct Kit use the following size standard peaks in their definitions:

GeneScan™ 500 LIZ® Size Standard peak sizes	GeneScan™ 600 LIZ® Size Standard v2.0 peak sizes
75, 100, 139, 150, 160, 200, 300, 350, 400, and 450	80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460

Note: The 250-nt and the 340-nt peak in the GeneScan™ 500 LIZ® Size Standard are not included in the size standard definition. These peaks can be used as an indicator of precision within a run.

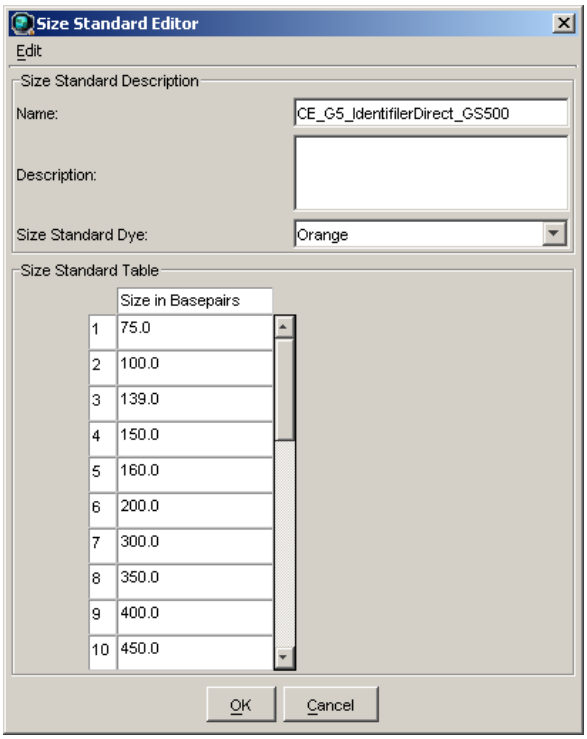
To create the size standard for the Identifiler® Direct Kit:

1. Select **Tools** ▸ **GeneMapper Manager** to open the GeneMapper Manager.



2. Select the **Size Standards** tab, then click **New**.

3. Enter a name as shown below or enter a name of your choosing. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the peak sizes specified in on page 47. The example below is for the GeneScan™ 500 LIZ® Size Standard.



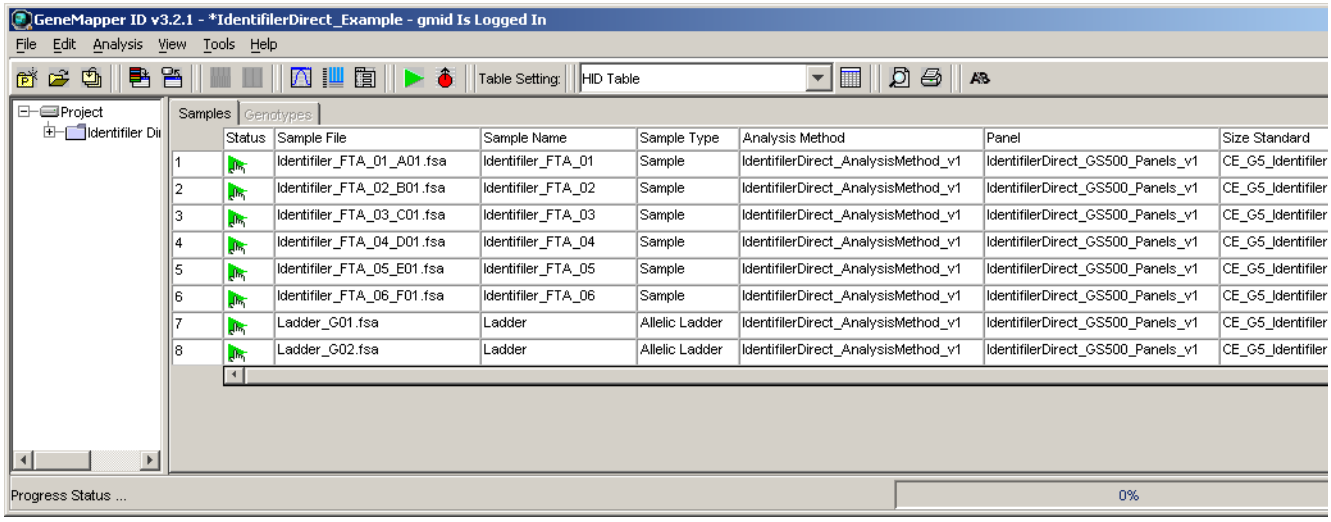
Analyze and edit sample files with GeneMapper® *ID* Software

1. In the Project window, select **File ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project. The names of the settings shown are the names suggested in the sections above. If you named the settings differently, select the names you specified.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	IdentifilerDirect_AnalysisMethod_v1 (or the name of the analysis method you created)
Panel	IdentifilerDirect_GS500_Panels_v1
Size Standard	CE_G5_IdentifilerDirect_GS500 (or the name of the size standard you created)

For more information about how the Size Caller works, refer to the *ABI PRISM® GeneScan® Analysis Software for the Windows NT® Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (Part no. 4335617). For additional information about size standards, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (Part no. 4338775).

3. Click ▶ (Analyze), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis. During a run:
   - The status bar displays the progress of analysis as both:
     - A completion bar extending to the right with the percentage completed indicated
     - With text messages on the left
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The Genotypes tab becomes available after analysis.



Examine and edit a project

You can display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data. These procedures start with the Samples tab of the Project window (assuming the analysis is complete).

For more information

For details about GeneMapper® *ID* Software features, allele filters, peak detection algorithms, and project editing, refer to:

- *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial (Part no. 4335523)*
- *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide (Part no. 4338775)*
- *Installation Procedures and New Features for GeneMapper® ID Software Software Version v3.2 User Bulletin* (Part no. 4352543)

Section 4.2 GeneMapper® *ID-X* Software

Overview of GeneMapper® *ID-X* Software

GeneMapper® *ID-X* Software is an automated genotyping software for forensic casework, databasing, and paternity data analysis.

After electrophoresis, the data collection software stores information for each sample in a .fsa or .hid file. Using GeneMapper® *ID-X* Software, you can then analyze and interpret the data from the .fsa files (GeneMapper® *ID-X* Software v1.0.1 or higher) or .hid files (GeneMapper® *ID-X* Software v1.2 or higher).

Instruments

Refer to "Instrument and software overview" on page 15 for a list of compatible instruments.

Before you start

When using GeneMapper® *ID-X* Software v1.0.1 or higher to perform human identification (HID) analysis with AmpFℓSTR® kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Perform the appropriate internal validation studies if you want to use multiple ladder samples in an analysis.

  For multiple ladder samples, the GeneMapper® *ID-X* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.

  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples to ensure proper allele calling.
- Alleles that are not in the AmpFℓSTR® Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, verify the sample result according to your laboratory protocol.

Set up GeneMapper® *ID-X* Software for data analysis

File names

The file names shown in this section may differ from the file names you see when you download or import files. If you need help determining the correct files to use, contact your local Life Technologies Human Identification representative, or go to **www.appliedbiosystems.com**.

Before using the software for the first time

Before you use GeneMapper® *ID-X* Software to analyze data files (GeneMapper® *ID-X* Software v1.0.1 or higher for .fsa files, GeneMapper® *ID-X* Software v1.2 or higher for .hid files):

- Import panels, bins, and marker stutter into the Panel Manager, as explained in "Import panels, bins, and marker stutter" on page 51.
- Create an analysis method, as explained in "Create an analysis method" on page 55.
- Create a size standard, as explained in "Create a size standard" on page 60.
- Define custom views of analysis tables.

  Refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Part no. 4375574) for more information.
- Define custom views of plots.

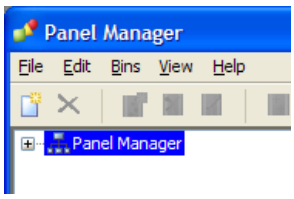
  Refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Part no. 4375574) for more information.

Import panels, bins, and marker stutter

To import the Identifiler® Direct Kit panel, bin set, and marker stutter from the Life Technologies web site into the GeneMapper® *ID-X* Software database:

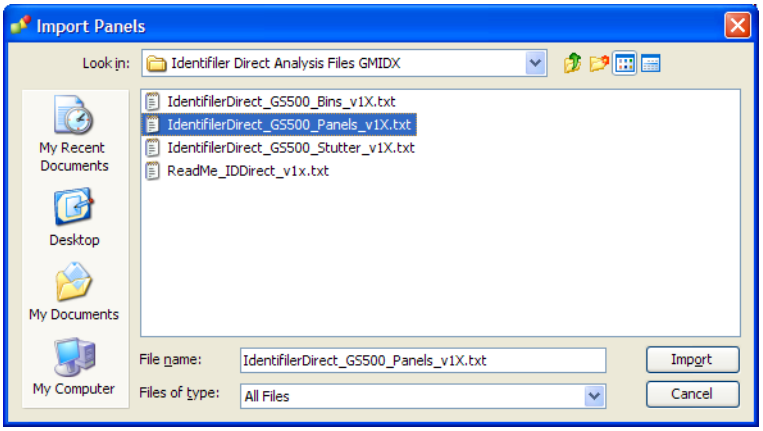
1. Download and open the file containing panels, bins, and marker stutter:
   a. From the Support menu of **www.lifetechnologies.com**, select **Support** ▸ **Software Downloads, Patches & Updates** ▸ GeneMapper® *ID-X* Software ▸ **Updates & Patches**, and download the file **Identifiler Direct Analysis Files GMIDX**.
   b. Unzip the file.
2. Start the GeneMapper® *ID-X* Software, then log in with the appropriate user name and password.

   IMPORTANT! For logon instructions, refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Part no. 4375574).
3. Select **Tools** ▸ **Panel Manager**.
4. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager** in the navigation pane.
   b. Select **File** ▸ **Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the **Identifiler Direct Analysis Files GMIDX** folder that you unzipped in step 1.



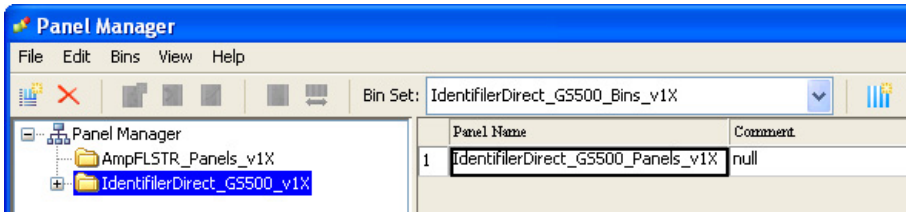
5. Select **IdentifilerDirect_GS500_v1X**, then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager "IdentifilerDirect_GS500_Panels_v1X". This folder contains the panel and associated markers.



6. Import IdentifilerDirect_GS500_Bins_v1X.txt:

   a. Select the **IdentifilerDirect_GS500_Panels_v1X** folder in the navigation pane.

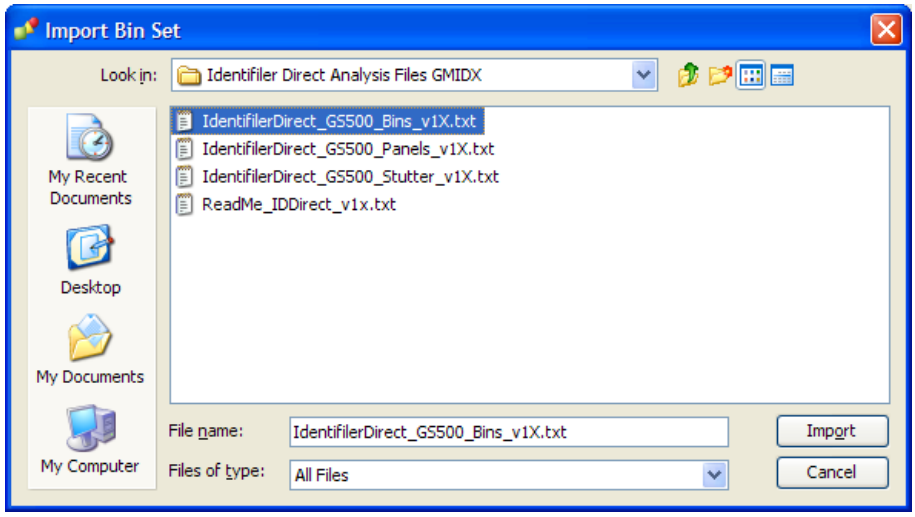


   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

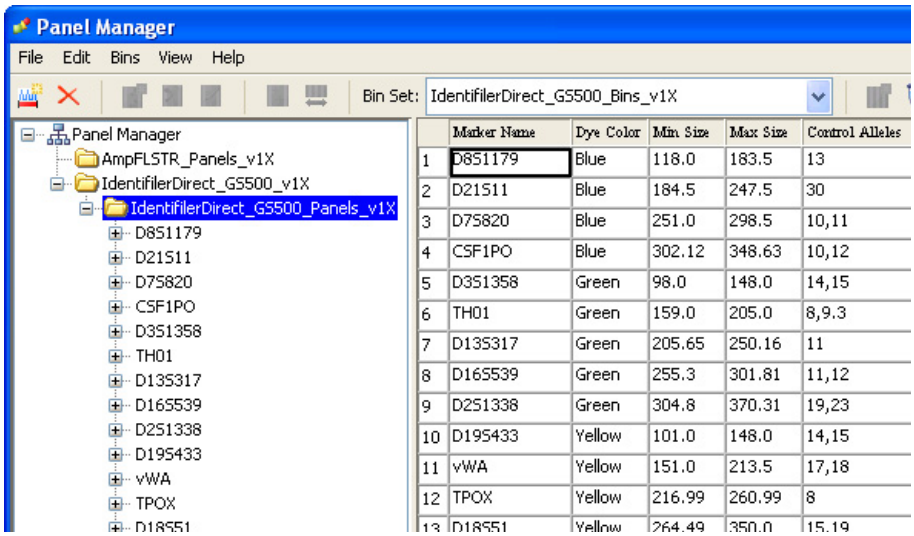
   c. Navigate to, then open the **Identifiler Direct Analysis Files GMIDX** folder.

   d. Select **IdentifilerDirect_GS500_Bins_v1X.txt**, then click **Import**.

   Note: Importing this file associates the bin set with the panels in the IdentifilerDirect_GS500_v1X folder.

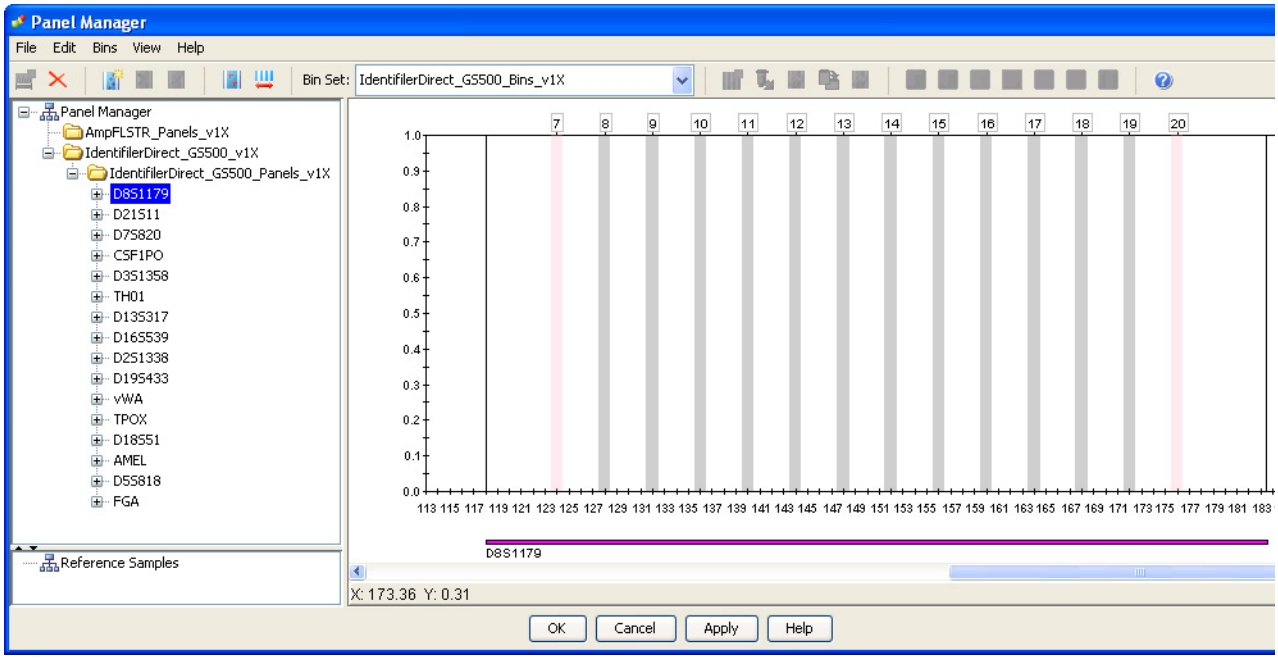


7. View the imported panels in the navigation pane:
   a. Double-click the **IdentifilerDirect_GS500_v1X** folder.
   b. Double-click the **IdentifilerDirect_GS500_Panels_v1X** folder to display the panel information in the right pane and the markers below it.

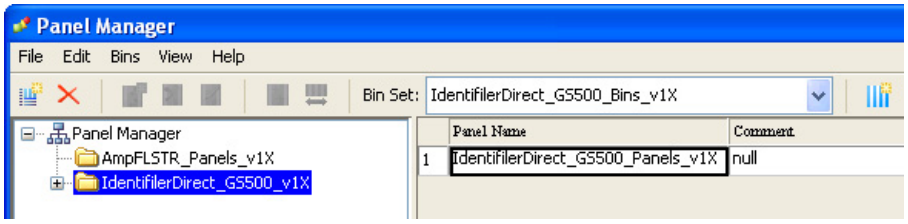


	Marker Name	Dye Color	Min Size	Max Size	Control Alleles
1	D8S1179	Blue	118.0	183.5	13
2	D21S11	Blue	184.5	247.5	30
3	D7S820	Blue	251.0	298.5	10,11
4	CSF1PO	Blue	302.12	348.63	10,12
5	D3S1358	Green	98.0	148.0	14,15
6	TH01	Green	159.0	205.0	8,9.3
7	D13S317	Green	205.65	250.16	11
8	D16S539	Green	255.3	301.81	11,12
9	D2S1338	Green	304.8	370.31	19,23
10	D19S433	Yellow	101.0	148.0	14,15
11	vWA	Yellow	151.0	213.5	17,18
12	TPOX	Yellow	216.99	260.99	8
13	D18S51	Yellow	264.49	350.0	15.19

8. Select **D8S1179** to display the Bin view for the marker in the right pane.

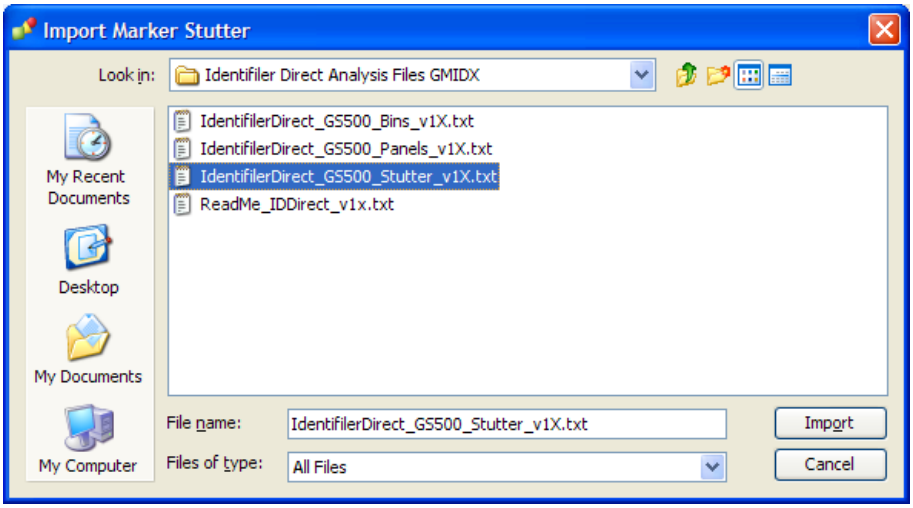


9. Import IdentifilerDirect_GS500_Stutter_v1X.txt:
   a. Select the **IdentifilerDirect_GS500_v1** folder in the navigation panel.



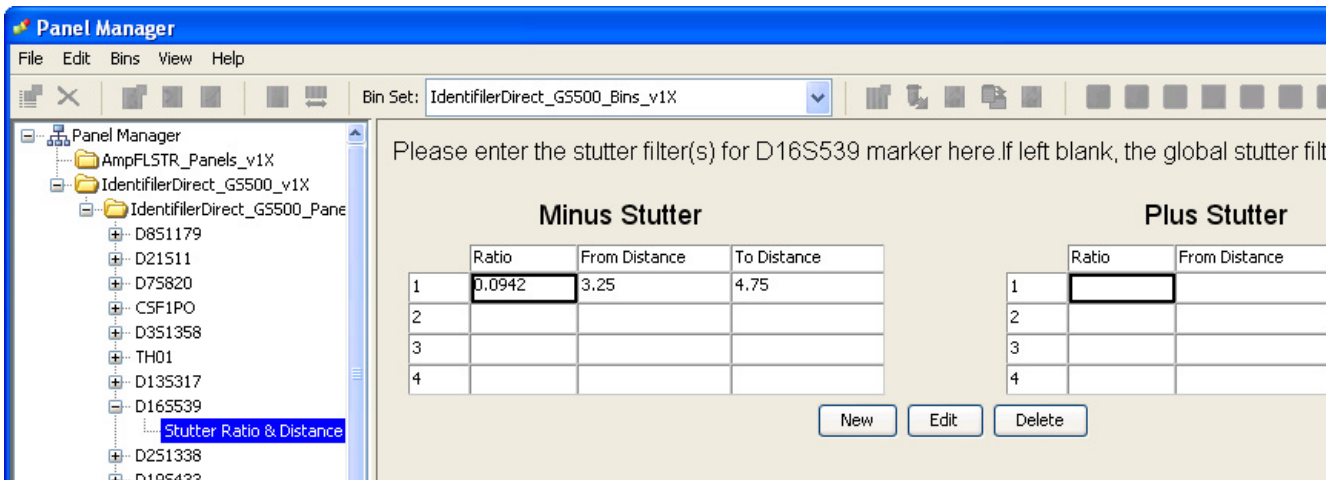
   b. Select File ▸ **Import Marker Stutter** to open the Import Marker Stutter dialog box.
   c. Navigate to, then open the **Identifiler Direct Analysis Files GMIDX** folder.
   d. Select **IdentifilerDirect_GS500_Stutter_v1X.txt**, then click **Import**.

   Note: Importing this file associates the marker stutter ratio with the bin set in the IdentifilerDirect_GS500_Bins_v1X folder.



10. View the imported marker stutters in the navigation pane:
    a. Select the **IdentifilerDirect_GS500_v1X** folder to display its list of markers in the right pane.
    b. Double-click the **IdentifilerDirect_GS500_v1X** folder to display its list of markers below it.

c. Double-click **D16S539** to display the Stutter Ratio & Distance view for the marker in the right pane.



11. Click **Apply**, then **OK** to add the Identifiler® Direct Kit panel, bin set, and marker stutter to the GeneMapper® *ID-X* Software database.

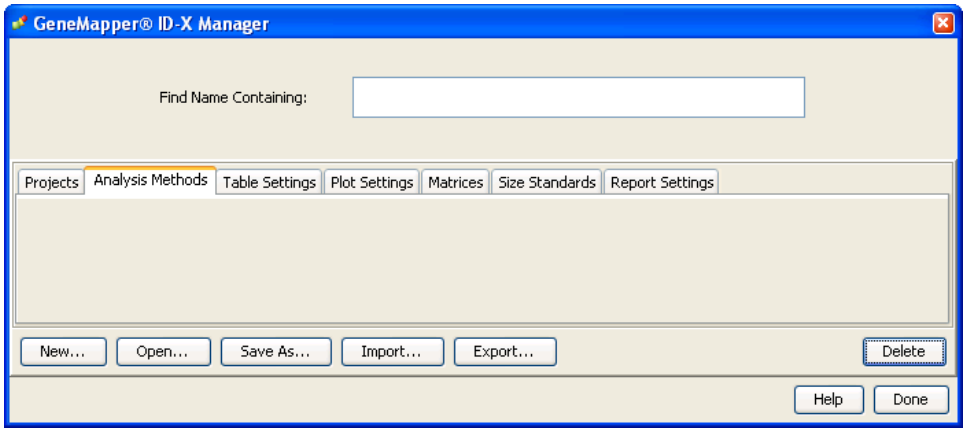
IMPORTANT! If you close the Panel Manager without clicking **Apply**, the panels, bin sets, and marker stutter will not be imported into the GeneMapper® *ID-X* Software database.

Create an analysis method

Use the following procedure to create an analysis method for the Identifiler® Direct Kit.

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method created for GeneMapper® *ID-X* Software version 1.2 is not compatible with earlier versions of GeneMapper® *ID-X* Software, or with GeneMapper® *ID* Software version 3.2.1.

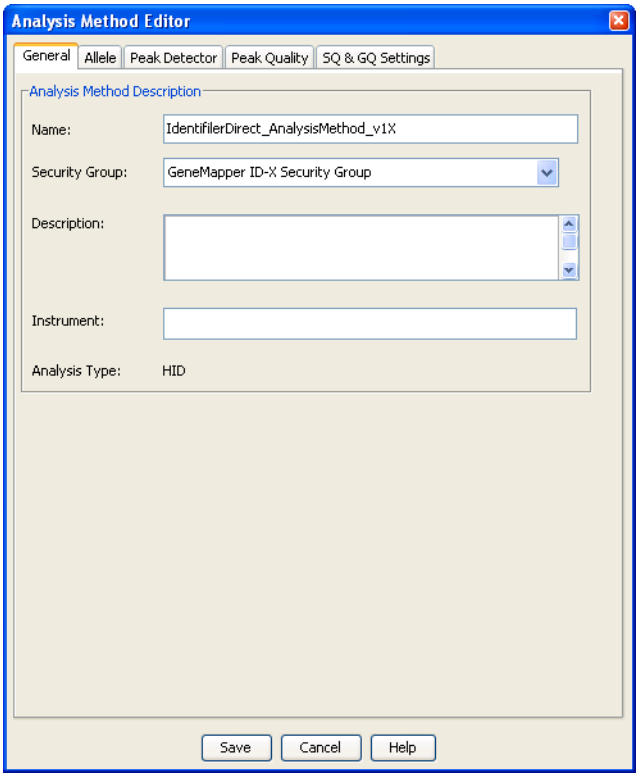
1. Select **Tools** ▸ **GeneMapper® *ID-X* Manager** to open the GeneMapper® *ID-X* Manager.



2. Select the **Analysis Methods** tab, then click **New** to open the Analysis Method Editor with the **General** tab selected.
3. Enter the settings shown in the figures on the following pages.

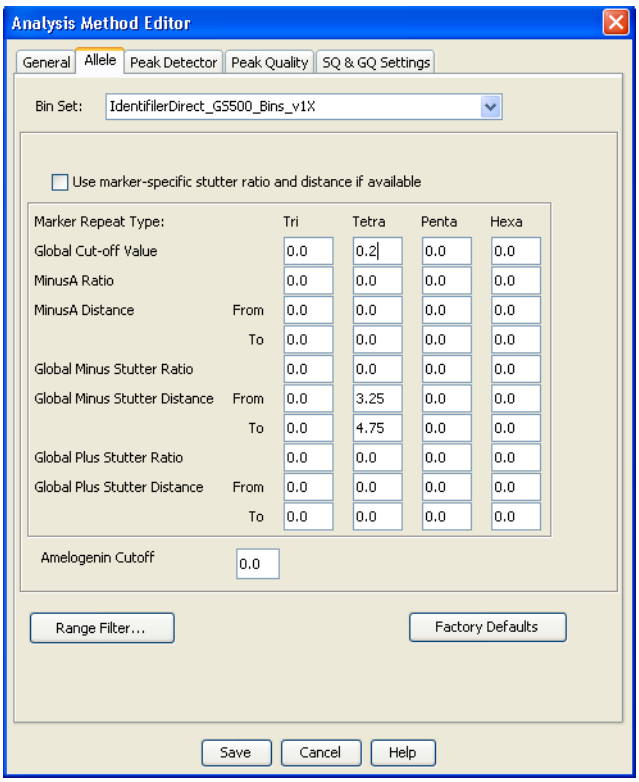
   Note: The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.
4. After you enter the settings on all tabs, click **Save**.

General tab settings



In the Name field, either type the name as shown or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the drop-down list. The Description and Instrument fields are optional.

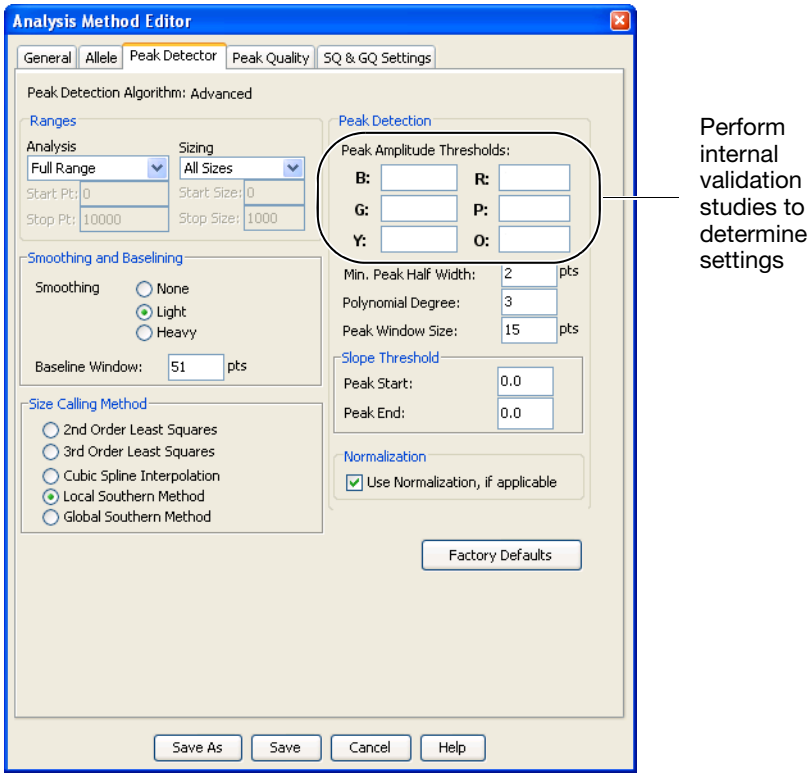
Allele tab settings



- In the Bin Set field, select the **IdentifilerDirect_GS500_Bins_v1X** bin set imported previously and configure the parameters as shown.
- GeneMapper® *ID-X* Software allows you to specify 4 types of marker repeat motifs: tri, tetra, penta and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the stutter ratio:
  - To apply the stutter ratios listed in the Allele tab for single-source data, deselect the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

    Note: Applying global stutter ratios may reduce the editing required for single-source sample data.
  - To apply the stutter ratios contained in the IdentifilerDirect_GS500_Stutter_v1X.txt file, select the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

Peak Detector tab settings



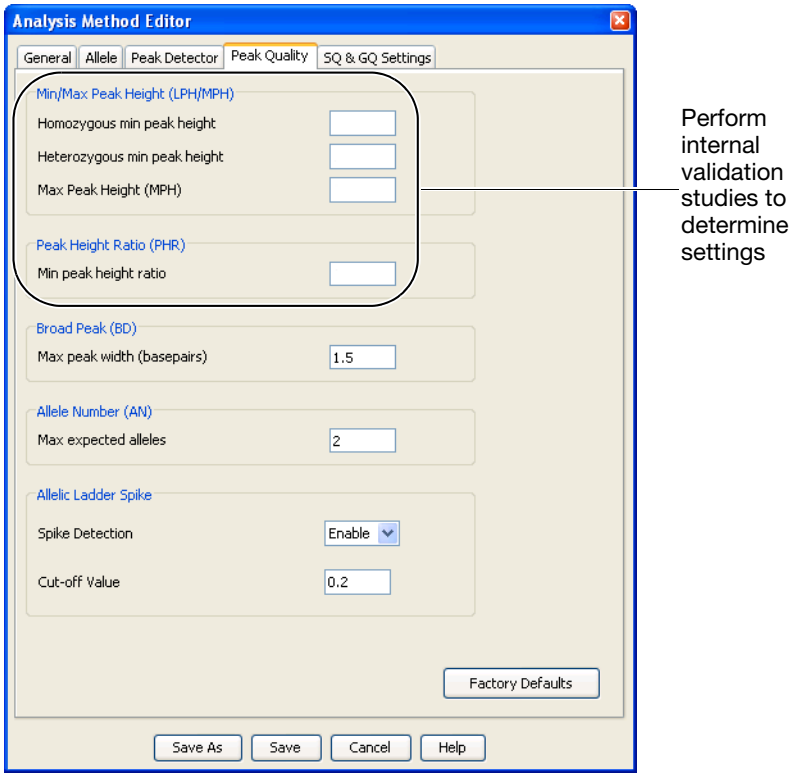
Perform internal validation studies to determine settings

IMPORTANT! Perform the appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of Identifiler® Direct Kit data.

Fields include:

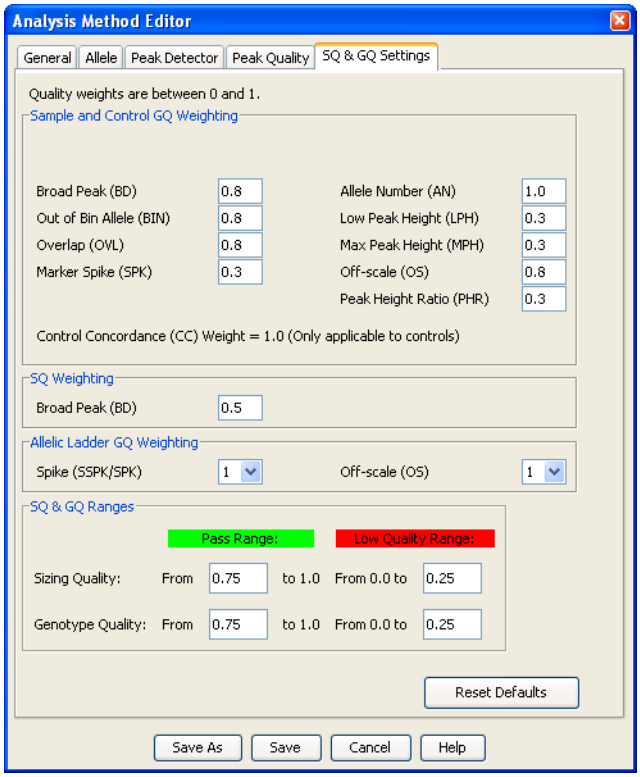
- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper® *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – This kit has been validated using the Local Southern sizing method. Before using alternative sizing methods, perform the appropriate internal validation studies.
- **Normalization** (v1.2 or higher) – For use with 3500 data. Perform internal validation studies to determine whether to use the Normalization feature for analysis of Identifiler® Direct Kit data.

Peak Quality tab settings



IMPORTANT! Perform the appropriate internal validation studies to determine the minimum heterozygous and homozygous minimum peak height thresholds, maximum peak height threshold, and the minimum peak height ratio threshold for interpretation of Identifiler® Direct Kit data.

SQ & GQ tab settings



IMPORTANT! The values shown are the software defaults and are the values we used during developmental validation. Perform appropriate internal validation studies to determine the appropriate values to use.

Create a size standard

The size standards for the Identifiler® Direct Kit uses the following size standard peaks in their definitions:

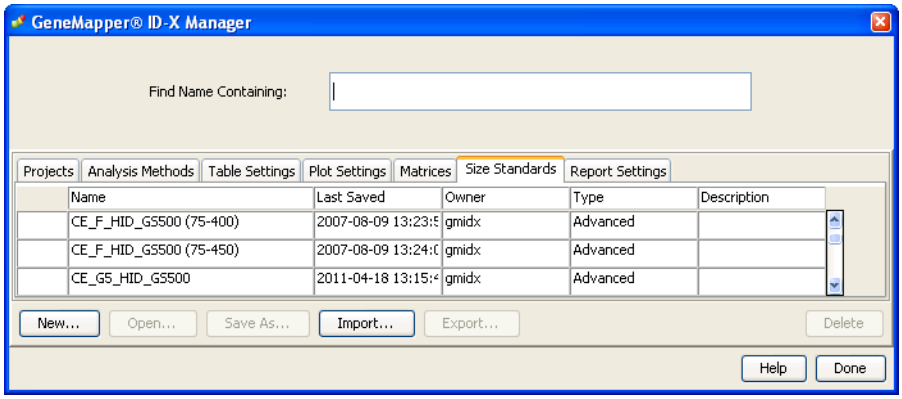
GeneScan™ 500 LIZ® Size Standard peak sizes	GeneScan™ 600 LIZ® Size Standard v2.0 peak sizes
75, 100, 139, 150, 160, 200, 300, 350, 400, and 450	80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460

Note: The 250-nt and the 340-nt peaks in the GeneScan™ 500 LIZ® Size Standard are not included in the size standard definition. These peaks can be used as an indicator of precision within a run.

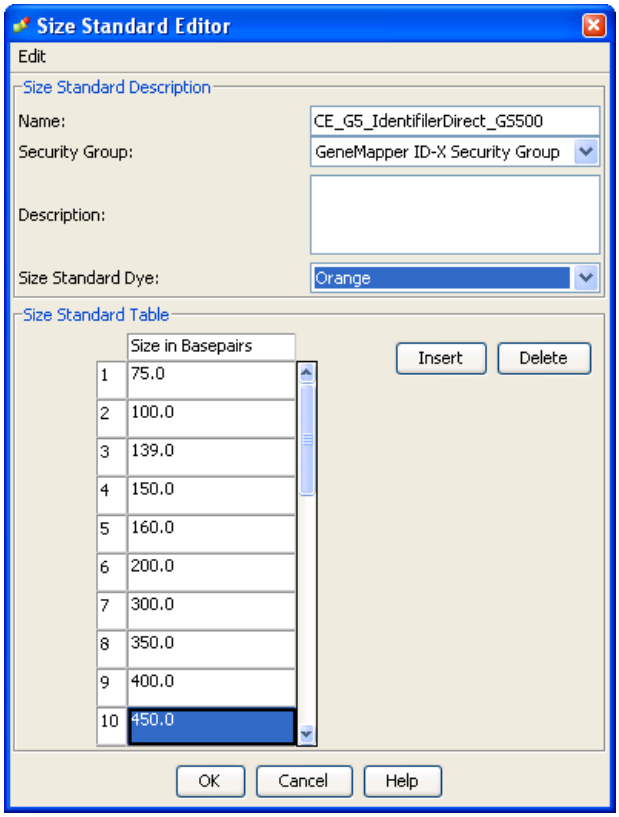
Use the following procedure to create the size standard for the Identifiler® Direct Kit.

1. Select **Tools** ▸ **GeneMapper® *ID-X* Manager** to open the GeneMapper® *ID-X* Manager.

2. Select the **Size Standards** tab, then click **New**.



3. Complete the Name field as shown below or with a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the drop-down list. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the peak sizes specified on page 60. The example below is for the GeneScan™ 500 LIZ® Size Standard.



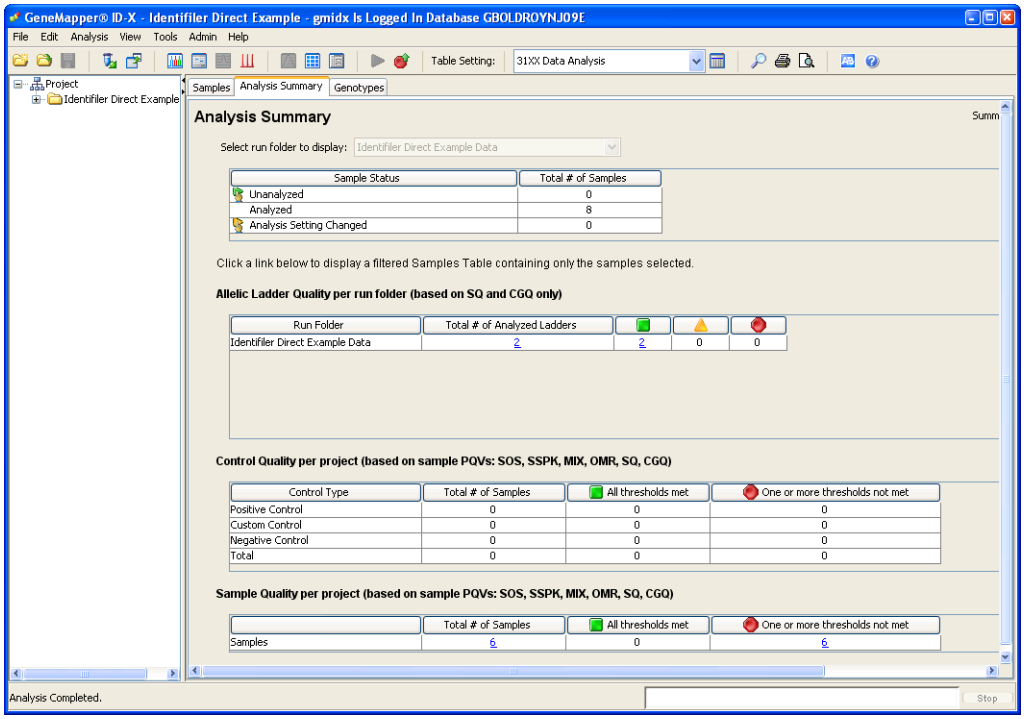
Analyze and edit sample files with GeneMapper® *ID-X* Software

1. In the Project window, select **File ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project. The names of the settings shown are the names suggested in the sections above. If you named the settings differently, select the names you specified.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	IdentifilerDirect_AnalysisMethod_v1X (or the name of the analysis method you created)
Panel	IdentifilerDirect_GS500_Panels_v1X
Size Standard	CE_G5_IdentifilerDirect_GS500 (or the name of the size standard you created)

   For more information about how the Size Caller works, or about size standards, refer to the *GeneMapper® ID-X Software v1.2 Reference Guide* (Part no. 4426481).
3. Click (Analyze), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis. During a run:
   - The status bar displays the progress of analysis as a completion bar extending to the right with the percentage completed indicated.
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The Analysis Summary tab is displayed upon completion of the analysis.

   The figure below shows the analysis summary window after analysis.



Examine and edit a project

You can display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data. These procedures start with the Analysis Summary tab of the Project window (assuming the analysis is complete).

For more information

For more information about any of these tasks, refer to:

- *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Part no. 4375574)
- *GeneMapper® ID-X Software Version 1.0 Quick Reference Guide* (Part no. 4375670)
- *GeneMapper® ID-X Software Version 1.0 Reference Guide* (Part no. 4375671)
- *GeneMapper® ID-X Software Version 1.1(Mixture Analysis) Getting Started Guide* (Part no. 4396773)
- *GeneMapper® ID-X Software Version 1.2 Reference Guide* (Part no. 4426481)
- *GeneMapper® ID-X Software Version 1.2 Quick Reference Guide* (Part no. 4426482)

5 Experiments and Results

Overview

This chapter provides results of the developmental validation experiments we performed using the Identifiler® Direct Kit for samples punched from FTA® cards.

Importance of validation

Validation of a DNA typing procedure for human identification applications is an evaluation of the procedure's efficiency, reliability, and performance characteristics. By challenging the procedure with samples commonly encountered in forensic and parentage laboratories, the validation process uncovers attributes and limitations which are critical for sound data interpretation in casework (Sparkes, Kimpton, Watson *et al.,* 1996; Sparkes, Kimpton, Gilbard *et al.,* 1996; Wallin *et al.,* 1998).

Experiment conditions

We performed experiments to evaluate the performance of the Identifiler® Direct Kit. according to the DNA Advisory Board (DAB) Quality Assurance Standards, effective October 1, 1998 (DNA Advisory Board, 1998). The DAB standards describe the quality assurance requirements that a laboratory should follow to ensure the quality and integrity of the data and competency of the laboratory.

Additional validation was performed according to the revised guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, July 10, 2003). Based on these guidelines, we conducted experiments that comply with guidelines 1.0 and 2.0 and its associated subsections. This DNA methodology is not novel. (Moretti *et al.,* 2001; Frank *et al.,* 2001; Wallin *et al.,* 2002; and Holt *et al.,* 2000).

This chapter discusses many of the experiments we performed and provides examples of results obtained. We chose conditions that produced optimum PCR product yield and that met reproducible performance standards. It is our opinion that while these experiments are not exhaustive, they are appropriate for a manufacturer of STR kits intended for forensic and/or parentage testing use. Each laboratory using the Identifiler® Direct Kit should perform their own internal validation studies.

Validation studies included testing on the following sample+substrate combinations:

- Unpurified, single-source blood or buccal samples on FTA® paper (treated paper substrate)
- Buccal samples on a Bode Buccal DNA Collector™ (untreated paper substrate)

Additional performance verification studies included testing on Copan 4N6 FLOQSwabs® (swab substrate).

We did not perform mixture or inhibition studies during the developmental validation of the Identifiler® Direct Kit because these tests are not relevant for the intended use of this chemistry.

Accuracy, precision, and reproducibility

SWGDAM guideline 1.2.1

"Developmental validation is the demonstration of the accuracy, precision, and reproducibility of a procedure by the manufacturer, technical organization, academic institution, government laboratory, or other party." (SWGDAM, July 2003)

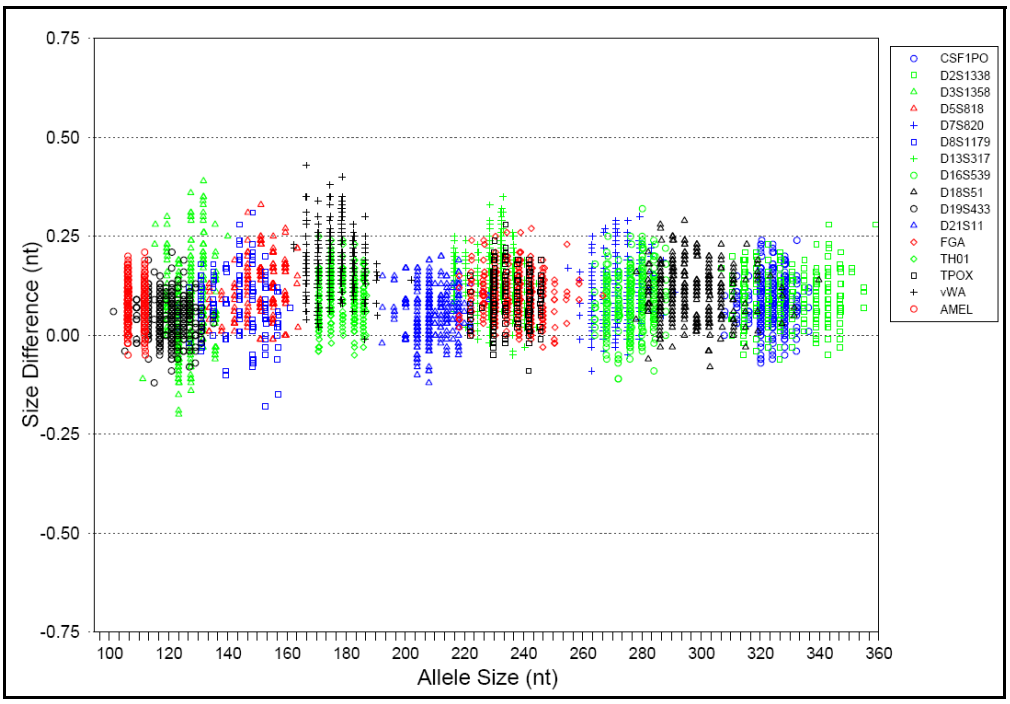
SWGDAM guideline 2.9

"The extent to which a given set of measurements of the same sample agree with their mean and the extent to which these measurements match the actual values being measured should be determined." (SWGDAM, July 2003)

Accuracy

Laser-induced fluorescence detection of length polymorphism at short tandem repeat loci is not a novel methodology (Holt *et al.*, 2000; and Wallin *et al.*, 2002). However, accuracy and reproducibility of Identifiler® Direct Kit profiles have been determined from various sample types. Figure 3 illustrates the size differences that are typically observed between sample alleles and allelic ladder alleles on the Applied Biosystems® 3130*xl* Genetic Analyzer with POP-4→ polymer. The x-axis in Figure 3 represents the nominal nucleotide sizes for the AmpFℓSTR® Identifiler® Direct Allelic Ladder. The dashed lines parallel to the x-axis represent ±0.25-nt windows. The y-axis represents the deviation of each sample allele size from the corresponding allelic ladder allele size. All sample alleles are within ±0.5 nt from a corresponding allele in the allelic ladder.

Figure 3 Size deviation of 200 blood samples on FTA® card analyzed on the Applied Biosystems 3130*xl* Genetic Analyzer



Precision and size windows

Sizing precision allows for determining accurate and reliable genotypes. Sizing precision was measured on the Applied Biosystems® 3130*xl* Genetic Analyzer. The recommended method for genotyping is to employ a ±0.5-nt "window" around the size obtained for each allele in the AmpFℓSTR® Identifiler® Direct Allelic Ladder. A ±0.5-nt window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside the specified window could be:

- An "off-ladder" allele, that is, an allele of a size that is not represented in the AmpFℓSTR® Identifiler® Direct Allelic Ladder

  or

- An allele that corresponds to an allelic ladder allele, but whose size falls just outside a window because of measurement error

The measurement error inherent in any sizing method can be defined by the degree of precision in sizing an allele multiple times. Precision is measured by calculating the standard deviation in the size values obtained for an allele that is run in several injections on a capillary instrument.

Table 3 on page 68 shows typical precision results obtained from five runs (16 capillaries/run) of the AmpFℓSTR® Identifiler® Direct Allelic Ladder on the Applied Biosystems® 3130*xl* Genetic Analyzer (36-cm capillary and POP-4® polymer) sized using the GeneScan™ 500 LIZ® Size Standard. The results were obtained within a consecutive set of injections on a single capillary array.

Sample alleles may occasionally size outside of the ±0.5-nt window for a respective allelic ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems having the smallest standard deviations in sizing. Figure 3 illustrates the tight clustering of allele sizes obtained on the Applied

Biosystems® 3130*xl* Genetic Analyzer, where the standard deviation in sizing is typically less than 0.15 nt. The instance of a sample allele sizing outside the ±0.5-nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

For sample alleles that do not size within a ±0.5-nt window, the PCR product must be rerun to distinguish between a true off-ladder allele versus measurement error of a sample allele that corresponds with an allele in the allelic ladder. Repeat analysis, when necessary, provides an added level of confidence to the final allele assignment.

GeneMapper® *ID* Software and GeneMapper® *ID-X* Software automatically flag sample alleles that do not size within the prescribed window around an allelic ladder allele by labelling the allele as OL (Off-ladder).

Maximum precision is obtained with a set of capillary injections on each of the supported platforms however the determined allele sizes will vary between the different platforms. Cross-platform sizing differences occur from a number of factors including type and concentration of polymer, run temperature, and electrophoresis conditions. Variations in sizing can also occur between runs on the same instrument and between runs on different instruments of the same platform type because of these factors.

We recommend strongly that the allele sizes obtained should be compared to the sizes obtained for known alleles in the AmpFℓSTR® Identifiler® Direct Allelic Ladder from the same run and then converted to genotypes (as described in "Before you start" on page 37 and 50). Refer to Table 3 for the results of five runs of the AmpFℓSTR® Identifiler® Direct Allelic Ladder. For more information on precision and genotyping, see Lazaruk *et al.*, 1998 and Mansfield *et al.*, 1998.

In Table 3, the mean sizes for all the alleles in each run (16 capillaries) were calculated. The mean range shown in the table represents the lowest- and highest-mean size values obtained across all five runs. Similarly, the standard deviation for the allele sizing was calculated for all the alleles in each run. The standard deviation range shown in Table 3 represents the lowest and highest standard deviation values obtained across all five runs.

Table 3 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Identifiler® Direct Allelic Ladder

Applied Biosystems® 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard deviation**
Amelogenin		
X	106.26–106.43	0.033–0.044
Y	111.92–112.06	0.032–0.046
CSF1PO		
6	304.04–304.20	0.038–0.053
7	308.09–308.26	0.033–0.052
8	312.15–312.32	0.038–0.047
9	316.20–316.37	0.033–0.048
10	320.24–320.42	0.027–0.051
11	324.30–324.45	0.033–0.055

Applied Biosystems® 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard deviation**
12	328.34–328.49	0.036–0.053
13	332.37–332.52	0.033–0.047
14	336.42–336.57	0.038–0.052
15	340.46–340.60	0.036–0.045
D13S317		
8	216.56–216.75	0.033–0.050
9	220.55–220.72	0.020–0.051
10	224.53–224.70	0.035–0.043
11	228.52–228.70	0.037–0.048
12	232.58–232.76	0.037–0.049
13	236.48–236.66	0.031–0.051
14	240.40–240.60	0.037–0.044
15	244.40–244.59	0.038–0.048
D16S539		
5	252.22–252.42	0.040–0.050
8	264.17–264.35	0.030–0.052
9	268.18–268.35	0.040–0.051
10	272.15–272.33	0.031–0.048
11	276.16–276.33	0.034–0.047
12	280.15–280.34	0.039–0.050
13	284.16–284.33	0.032–0.052
14	288.17–288.33	0.029–0.058
15	292.17–292.36	0.037–0.055
D18S51		
7	261.88–261.98	0.028–0.045
9	269.99–270.12	0.039–0.058
10	274.08–274.20	0.031–0.045
10.2	276.08–276.20	0.029–0.054
11	278.15–278.28	0.040–0.047
12	282.22–282.35	0.036–0.049
13	286.27–286.40	0.038–0.053
13.2	288.28–288.42	0.040–0.050
14	290.37–290.50	0.033–0.049
14.2	292.39–292.50	0.037–0.053
15	294.47–294.60	0.038–0.050
16	298.55–298.70	0.041–0.053

Applied Biosystems® 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard deviation**
17	302.68–302.82	0.034–0.052
18	306.82–306.99	0.042–0.053
19	310.96–311.11	0.043–0.060
20	315.10–315.25	0.031–0.048
21	319.23–319.38	0.031–0.049
22	323.42–323.57	0.038–0.054
23	327.48–327.63	0.043–0.055
24	331.59–331.74	0.031–0.052
25	335.69–335.83	0.029–0.052
26	339.81–339.96	0.044–0.052
27	343.92–344.04	0.037–0.051
D19S433		
9	101.38–101.46	0.032–0.039
10	105.28–105.36	0.030–0.036
11	109.20–109.28	0.027–0.042
12	113.14–113.22	0.028–0.038
12.2	115.15–115.21	0.032–0.038
13	117.11–117.17	0.030–0.045
13.2	119.11–119.17	0.028–0.038
14	121.07–121.14	0.022–0.045
14.2	123.10–123.17	0.035–0.047
15	125.09–125.13	0.031–0.048
15.2	127.12–127.16	0.026–0.045
16	129.11–129.16	0.034–0.044
16.2	131.17–131.20	0.028–0.044
17	133.17–133.22	0.033–0.044
17.2	135.24–135.27	0.022–0.043
D21S11		
24	184.40–184.51	0.035–0.042
24.2	186.39–186.50	0.023–0.043
25	188.35–188.44	0.025–0.040
26	192.30–192.39	0.029–0.043
27	196.27–196.33	0.026–0.042
28	200.14–200.21	0.039–0.043
28.2	202.11–202.18	0.028–0.042
29	204.08–204.16	0.031–0.041

Applied Biosystems® 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard deviation**
29.2	206.14–206.21	0.031–0.041
30	208.10–208.17	0.024–0.039
30.2	210.08–210.15	0.019–0.040
31	212.09–212.16	0.028–0.036
31.2	214.06–214.13	0.025–0.041
32	216.07–216.15	0.032–0.045
32.2	218.04–218.12	0.030–0.038
33	220.06–220.14	0.022–0.042
33.2	222.01–222.07	0.029–0.045
34	224.13–224.21	0.020–0.041
34.2	226.02–226.11	0.030–0.042
35	228.10–228.18	0.027–0.047
35.2	230.02–230.10	0.036–0.052
36	232.01–232.10	0.032–0.046
37	236.07–236.15	0.030–0.040
38	240.00–240.09	0.036–0.045
D2S1338		
15	306.40–306.56	0.032–0.056
16	310.49–310.64	0.036–0.049
17	314.56–314.72	0.034–0.048
18	318.62–318.77	0.038–0.040
19	322.69–322.84	0.025–0.044
20	326.74–326.89	0.035–0.049
21	330.81–330.95	0.030–0.042
22	334.87–335.00	0.029–0.047
23	338.90–339.05	0.039–0.052
24	342.94–343.08	0.039–0.047
25	346.99–347.13	0.031–0.050
26	350.99–351.13	0.040–0.051
27	354.94–355.06	0.031–0.050
28	359.08–359.21	0.031–0.054

Applied Biosystems® 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard deviation**
D3S1358		
12	111.35–111.49	0.034–0.052
13	115.45–115.58	0.034–0.046
14	119.44–119.58	0.034–0.047
15	123.37–123.49	0.035–0.053
16	127.55–127.67	0.033–0.051
17	131.74–131.86	0.029–0.048
18	135.85–135.96	0.035–0.050
19	139.96–140.07	0.036–0.056
D5S818		
7	133.85–133.95	0.037–0.048
8	137.96–138.06	0.040–0.046
9	142.31–142.42	0.032–0.045
10	146.78–146.89	0.033–0.044
11	151.13–151.26	0.032–0.043
12	155.36–155.50	0.027–0.042
13	159.51–159.67	0.020–0.045
14	163.57–163.73	0.032–0.044
15	167.60–167.76	0.030–0.055
16	171.63–171.77	0.036–0.049
D7S820		
6	255.09–255.23	0.031–0.047
7	259.11–259.25	0.038–0.048
8	263.13–263.27	0.036–0.049
9	267.16–267.29	0.029–0.041
10	271.20–271.32	0.041–0.048
11	275.23–275.37	0.032–0.051
12	279.26–279.40	0.037–0.047
13	283.28–283.43	0.035–0.049
14	287.32–287.45	0.043–0.052
15	291.35–291.49	0.037–0.053
D8S1179		
8	122.84–122.95	0.030–0.046
9	126.91–127.01	0.027–0.053
10	131.01–131.10	0.031–0.052
11	135.14–135.24	0.037–0.051

Applied Biosystems® 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard deviation**
12	139.33–139.43	0.029–0.059
13	143.90–144.02	0.027–0.045
14	148.36–148.48	0.034–0.045
15	152.70–152.82	0.022–0.044
16	156.93–157.09	0.026–0.041
17	161.08–161.24	0.026–0.046
18	165.14–165.33	0.035–0.056
19	169.22–169.40	0.035–0.056
FGA		
17	214.31–214.49	0.035–0.046
18	218.33–218.5	0.037–0.046
19	222.38–222.56	0.020–0.047
20	226.40–226.58	0.036–0.046
21	230.42–230.60	0.032–0.046
22	234.46–234.65	0.033–0.047
23	238.49–238.69	0.038–0.048
24	242.54–242.73	0.038–0.054
25	246.57–246.78	0.033–0.050
26	250.62–250.82	0.039–0.059
26.2	252.63–252.82	0.040–0.045
27	254.63–254.82	0.035–0.053
28	258.69–258.89	0.038–0.051
29	262.75–262.95	0.045–0.053
30	266.81–267.04	0.033–0.054
30.2	268.66–268.85	0.042–0.062
31.2	272.72–272.93	0.039–0.052
32.2	276.78–277.01	0.037–0.055
33.2	280.85–281.07	0.044–0.053
42.2	317.96–318.20	0.042–0.057
43.2	322.08–322.31	0.051–0.056
44.2	326.18–326.43	0.039–0.059
45.2	330.33–330.55	0.046–0.060
46.2	334.34–334.56	0.039–0.047
47.2	338.43–338.65	0.047–0.056
48.2	342.59–342.80	0.047–0.064
50.2	350.71–350.91	0.040–0.053

Applied Biosystems® 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard deviation**
51.2	354.67–354.87	0.048–0.058
TH01		
4	162.79–162.92	0.034–0.054
5	166.84–166.97	0.034–0.048
6	170.88–171.00	0.030–0.047
7	174.88–175.01	0.028–0.046
8	178.89–179.01	0.031–0.045
9	182.87–182.98	0.031–0.042
9.3	185.90–186.02	0.025–0.049
10	186.83–186.94	0.035–0.047
11	190.79–190.89	0.025–0.046
12	201.62–201.71	0.034–0.045
TPOX		
6	221.96–222.07	0.030–0.043
7	225.93–226.04	0.035–0.044
8	229.90–230.01	0.027–0.043
9	233.86–233.98	0.032–0.039
10	237.86–237.98	0.023–0.049
11	241.83–241.96	0.028–0.037
12	245.84–245.95	0.032–0.043
13	249.83–249.93	0.027–0.044
vWA		
11	154.16–154.27	0.025–0.044
12	158.30–158.44	0.029–0.054
13	162.40–162.54	0.034–0.045
14	166.62–166.78	0.029–0.048
15	170.56–170.70	0.028–0.046
16	174.57–174.71	0.028–0.045
17	178.56–178.71	0.028–0.045
18	182.51–182.66	0.032–0.044
19	186.48–186.60	0.031–0.045
20	190.41–190.53	0.026–0.043
21	194.29–194.43	0.032–0.044
22	198.21–198.33	0.025–0.043
23	202.06–202.18	0.034–0.040
24	206.38–206.48	0.031–0.040

Extra peaks in the electropherogram

Causes of extra peaks

Peaks other than the target alleles may be detected on the electropherogram. Causes for the appearance of extra peaks include stutter products, incomplete 3′ A nucleotide addition (at the n-1 position), dye artifacts, and mixed DNA samples (see DAB Standard 8.1.2.2).

Stutter products

A stutter is a well-characterized PCR artifact that refers to the appearance of a minor peak one repeat unit smaller (or less frequently, one repeat larger) than the major STR product (Butler, 2005; Mulero *et al.*, 2006). Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al.*, 1996).

The proportion of the stutter product relative to the main allele (percent stutter) is measured by dividing the height of the stutter peak by the height of the main allele peak. Peak heights were measured for amplified samples at the loci used in the Identifiler® Direct Kit:

- **Treated paper workflow**: 370 blood samples on FTA® card and 299 buccal samples on Indicating FTA® cards
- **Untreated paper workflow**: 370 buccal samples on Bode Buccal DNA Collectors

All data were generated on the Applied Biosystems 3130*xl* Genetic Analyzer.

Some conclusions from these measurements and observations are:

- For each Identifiler® Direct Kit locus, the percent stutter generally increases with allele length, as shown in
  - **Treated paper workflow**: Figure 4 through Figure 7 on page 76 through page 77
  - **Untreated paper workflow:** Figure 8 through Figure 11 on page 78 through page 79
- Smaller alleles display a lower level of stutter relative to the longer alleles within each locus.
- Each allele within a locus displays a percent stutter that is consistent.
- The stutter value for each locus shown for the treated paper workflow in Table 4 on page 80 was determined by taking the mean plus three times the standard deviation. These values are the stutter filter percentages in the Identifiler® Direct stutter file and will be used during the filtering step in GeneMapper® *ID* Software or GeneMapper® *ID-X* Software. Peaks in the stutter position that are above the stutter filter percentage will not be filtered. Peaks in the stutter position that have not been filtered and remain labeled can be further evaluated.
- Stutter percentages generated using the untreated paper workflow were calculated on a different, smaller data set than was used for the original stutter calculations. We used the stutter values of the most common alleles at each locus to compare the data sets. There was no significant difference in the stutter values (mean plus three times the standard deviation) for the individual loci with the exception of D3S1358 (0.7%), D7S820 (0.4%), D16S539 (0.2%) and FGA (1.2%). For D3S1358, D7S820, and FGA, the stutter values were slightly lower than the original stutter values calculated for punches from FTA® cards processed with the

Identifiler® Direct Kit. The D16S539 stutter percentage (mean plus three times the standard deviation) was slightly higher than the original stutter value. You should evaluate the impact of sample type on stutter percentages when implementing a direct amplification system.

- The measurement of percent stutter for allele peaks that are off-scale may be unusually high. Off-scale peaks were not included in the evaluation of stutter characterized here.

Figure 4 Treated paper workflow: FTA® card sample stutter percentages for D8S1179, D21S11, D7S820, and CSF1PO loci (red = blood samples; blue = buccal samples)

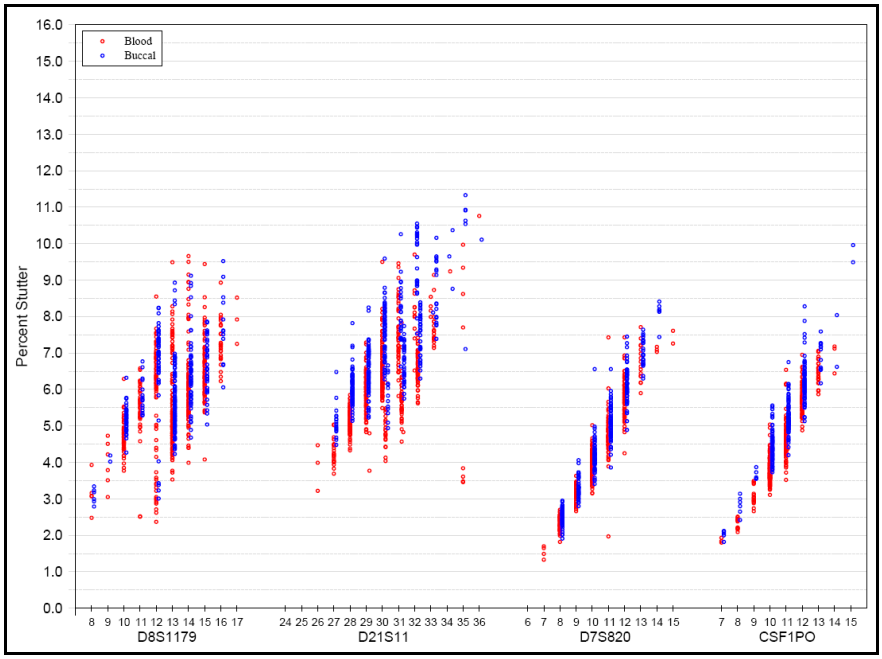


Figure 5 Treated paper workflow: FTA® card sample stutter percentages for D3S1358, TH01, D13S317, D16S539, and D2S1338 loci (red = blood samples; blue = buccal samples)

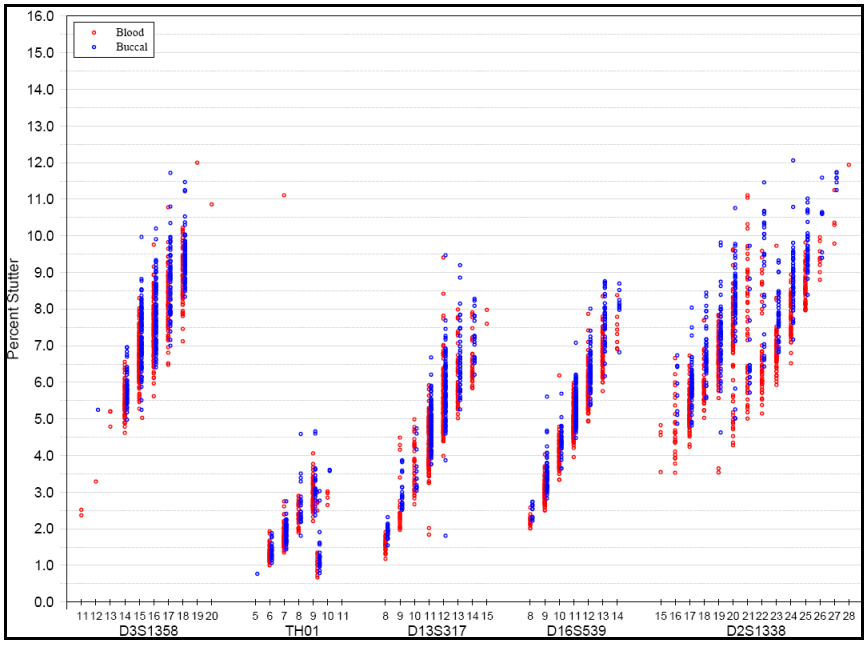


Figure 6 Treated paper workflow: FTA® card sample stutter percentages for D19S433, vWA, TPOX, and D18S51 loci (red = blood samples; blue = buccal samples)

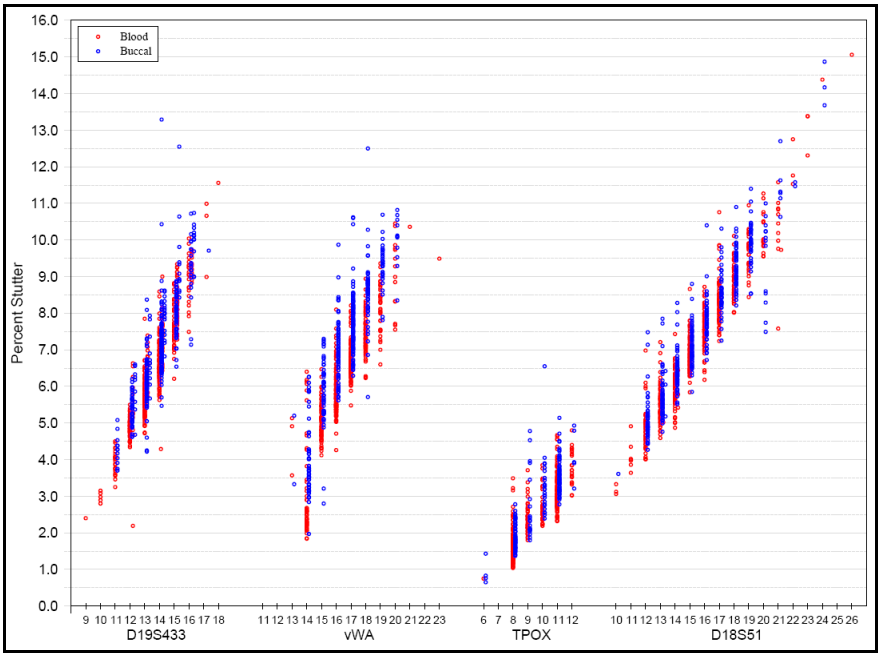


Figure 7 Treated paper workflow: FTA® card sample stutter percentages for D5S818 and FGA loci (red = blood samples; blue = buccal samples)

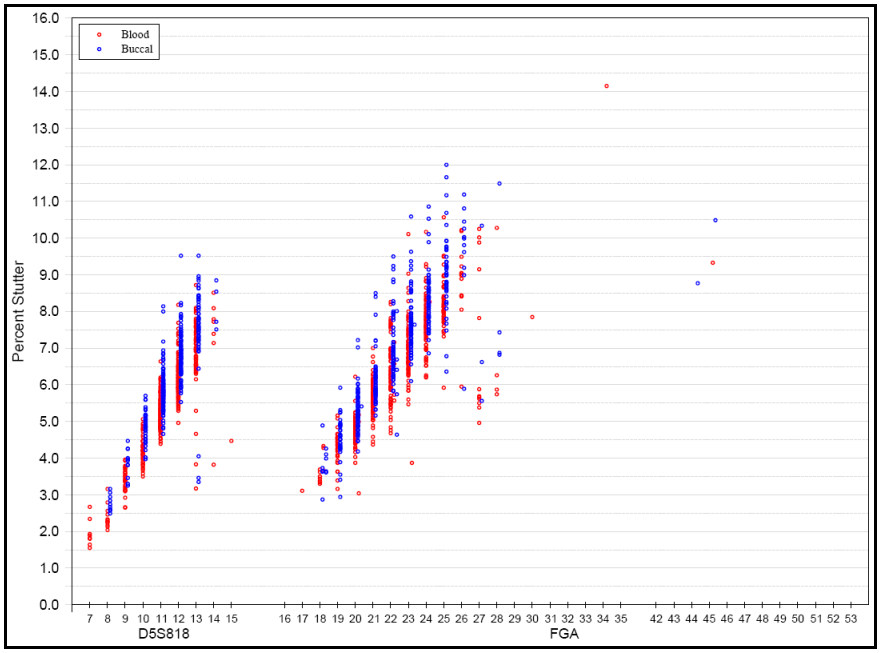


Figure 8 Untreated paper workflow: Bode Buccal DNA Collector™ sample stutter percentages for D8S1179, D21S11, D7S820, and CSF1PO loci

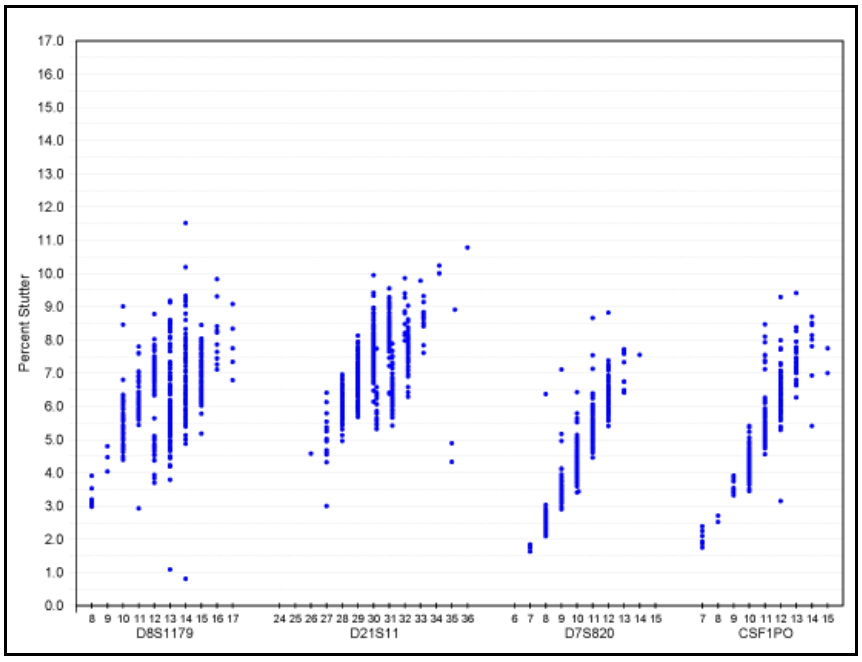


Figure 9 Untreated paper workflow: Bode Buccal DNA Collector™ sample stutter percentages for D3S1358, TH01, D13S317, D16S539, and D2S1338 loci

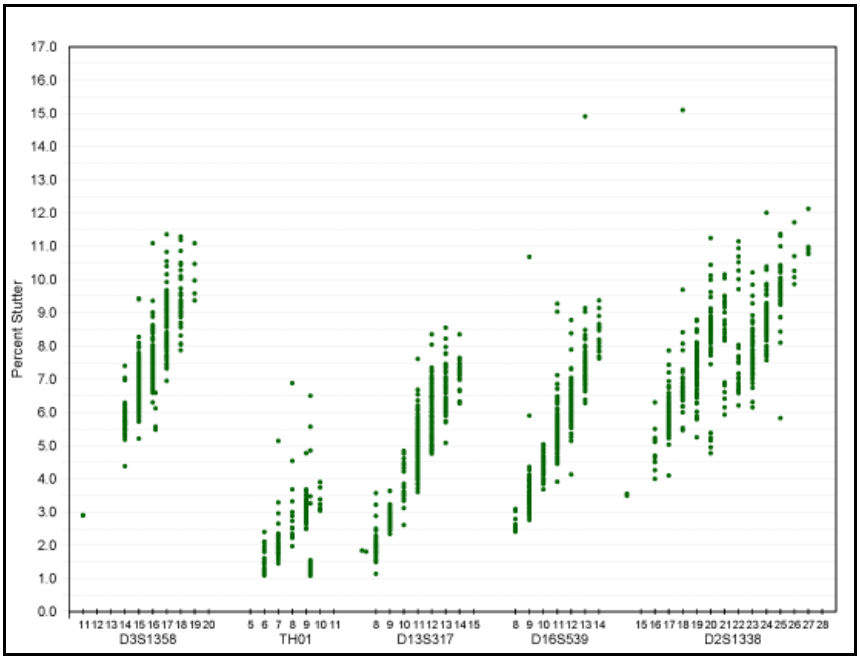


Figure 10 Untreated paper workflow: Bode Buccal DNA Collector™ sample stutter percentages for D19S433, vWA, TPOX, and D18S51 loci

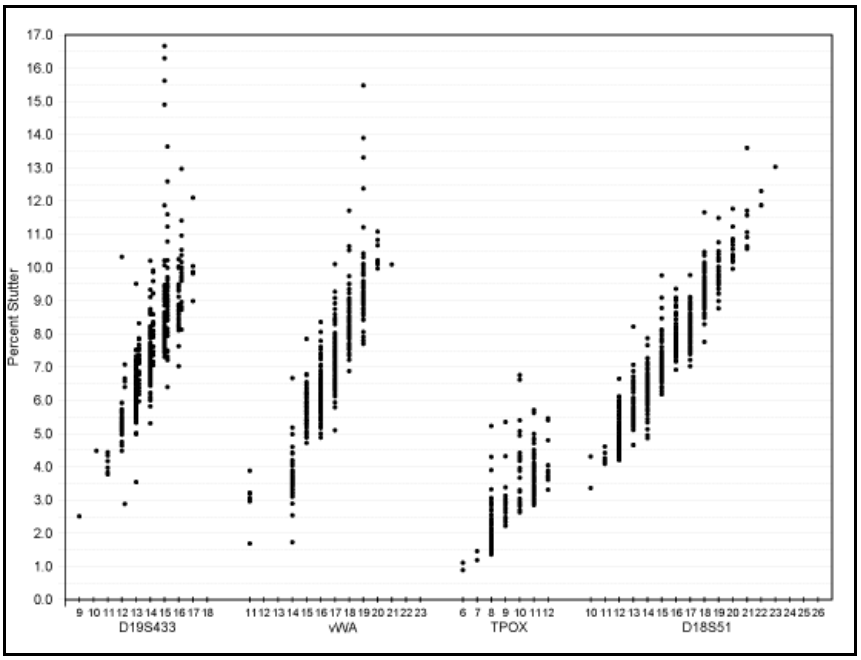


Figure 11 Untreated paper workflow: Bode Buccal DNA Collector™ sample stutter percentages for D5S818 and FGA loci (red = blood samples; blue = buccal samples)

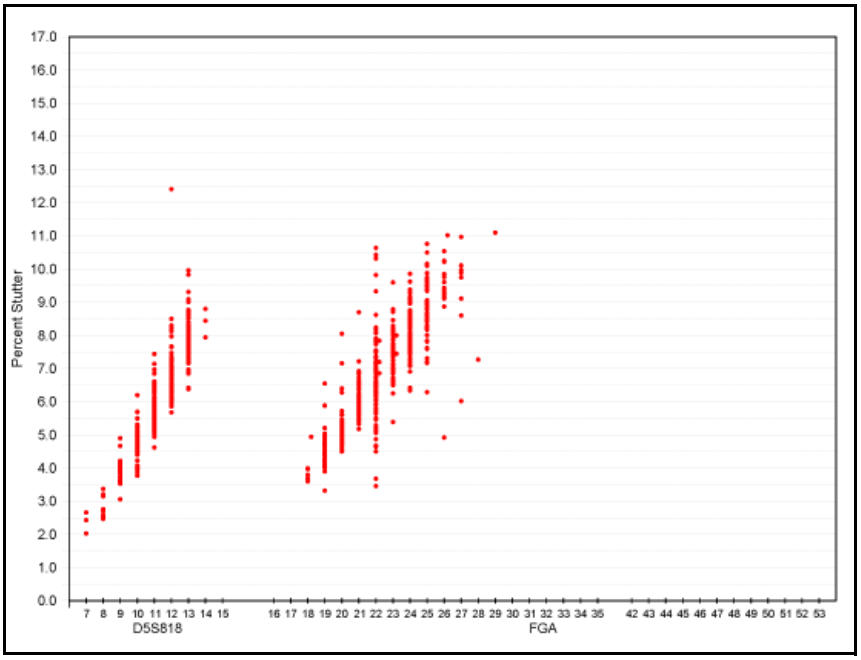


Table 4 Treated paper workflow: FTA® card sample marker-specific stutter filter percentages for Identifiler® Direct Kit loci

Locus	% Stutter†
CSF1PO	8.48
D13S317	9.39
D16S539	9.42
D18S51	12.89
D19S433	11.15
D21S11	10.42
D2S1338	11.77
D3S1358	11.45
D5S818	9.89
D7S820	8.60
D8S1179	9.54
FGA	11.62
TH01	4.76
TPOX	5.27
vWA	11.99

† These percentages are used as stutter filters in GeneMapper® *ID* Software IdentifilerDirect_GS500_Panels_v1 and GeneMapper® *ID-X* Software IdentifilerDirect_GS500_Stutter_v1X.txt.

Addition of 3′ A nucleotide

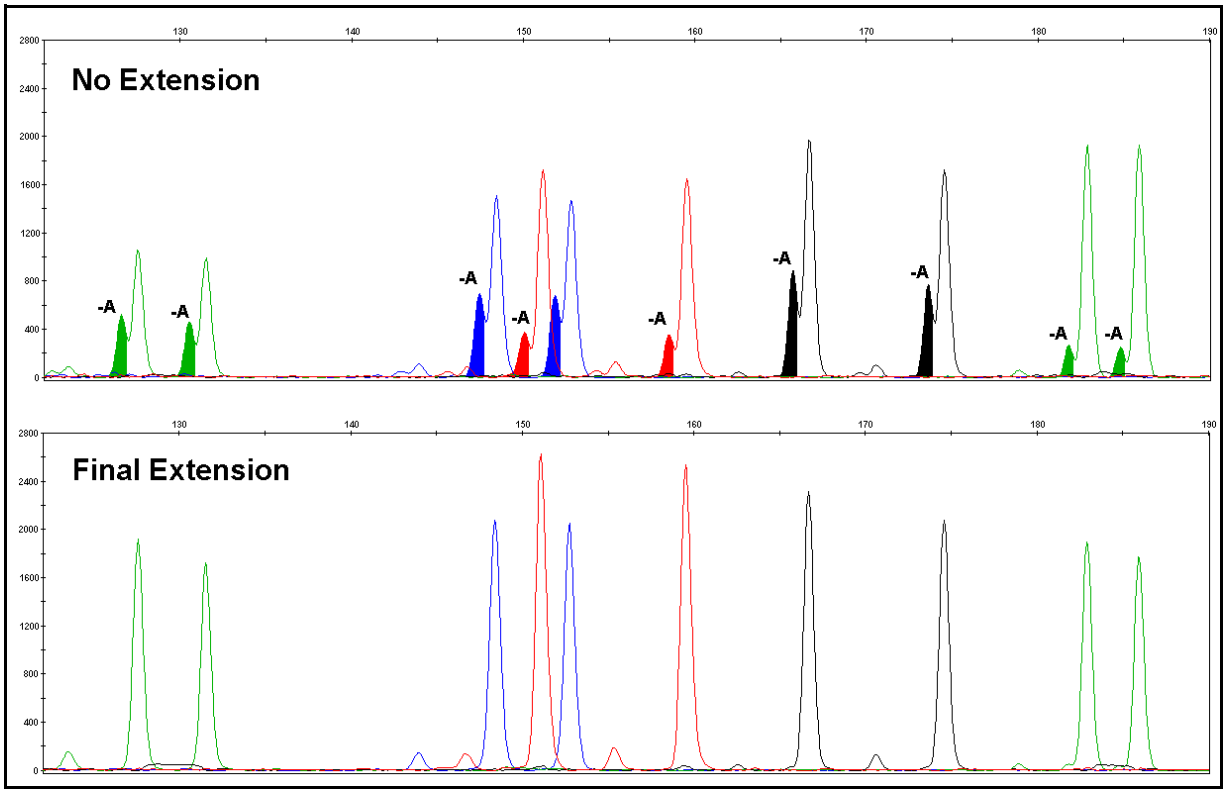
AmpliTaq Gold® enzyme, like many other DNA polymerases, can catalyze the addition of a single nucleotide (predominately adenosine) to the 3′ ends of double-stranded PCR products (Clark, 1988; Magnuson *et al.*, 1996). This nontemplate addition results in a PCR product that is one nucleotide longer than the actual target sequence. The PCR product with the extra nucleotide is referred to as the "+A" form.

The efficiency of +A addition is related to the particular sequence of the DNA at the 3′ end of the PCR product. The AmpFℓSTR® Identifiler® Direct PCR Amplification Kit includes two main design features that promote maximum +A addition:

- The primer sequences have been optimized to encourage +A addition.
- The final extension step is 60°C for 25 min.

The final extension step gives the AmpliTaq Gold® DNA polymerase additional time to complete +A addition to all double-stranded PCR products. STR systems (where each allele is represented by two peaks that are one nucleotide apart) that have not been optimized for +A addition may have "split peaks."

Figure 12 Omitting the final extension step results in split peaks due to incomplete A nucleotide addition. Data are from an Applied Biosystems 3130*xl* Genetic Analyzer using the AmpFℓSTR® Identifiler® Direct Kit.



Artifacts

Artifacts and anomalies are seen in all molecular biological systems. Artifacts are typically reproducible while anomalies are non-reproducible, intermittent occurrences that are not observed consistently in a system (for example, spikes and baseline noise). Reproducible artifacts have not been seen in data produced on the genetic analyzers used during developmental validation of the Identifiler® Direct Kit.

Characterization of loci

SWGDAM guideline 2.1

"The basic characteristics of a genetic marker must be determined and documented." (SWGDAM, July 2003)

This section describes basic characteristics of the 15 loci and the sex-determining marker, Amelogenin that are amplified with the Identifiler® Direct Kit. These loci have been extensively characterized by other laboratories.

Nature of the polymorphisms

The primers for the Amelogenin locus flank a 6-nucleotide deletion within intron 1 of the X homologue. Amplification results in 107-nt and 113-nt products from the X and Y chromosomes, respectively. (Sizes are the actual nucleotide size according to sequencing results, including 3′ A nucleotide addition.) The remaining Identifiler® Direct Kit loci are all tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of a particular locus result from differences in the number of 4-nt repeat units.

We have subjected to sequencing all the alleles in the AmpFℓSTR® Identifiler® Direct Allelic Ladder. In addition, other groups in the scientific community have sequenced alleles at some of these loci. Among the various sources of sequence data on the Identifiler® Direct Kit loci, there is consensus on the repeat patterns and structure of the STRs.

Mapping

The Identifiler® Direct Kit loci have been mapped, and the chromosomal locations have been published (Nakahori *et al.*, 1991; Edwards *et al.*, 1992; Kimpton *et al.*, 1992; Mills *et al.*, 1992; Sharma and Litt, 1992; Li *et al.*, 1993; Straub *et al.*, 1993; Barber and Parkin, 1996).

Species specificity

SWGDAM Guideline 2.2

"For techniques designed to type human DNA, the potential to detect DNA from forensically relevant nonhuman species should be evaluated." (SWGDAM, July 2003)

The Identifiler® Direct Kit provides the required specificity for detecting primate alleles. Other species do not amplify for the loci tested.

Nonhuman studies

Nonhuman DNA may be present in forensic casework samples. The data from Identifiler® Direct Kit experiments on nonhuman DNA sources are shown in Figure 13.

Figure 13 Representative electropherograms from a species-specificity study including positive and non-template controls (NTC)

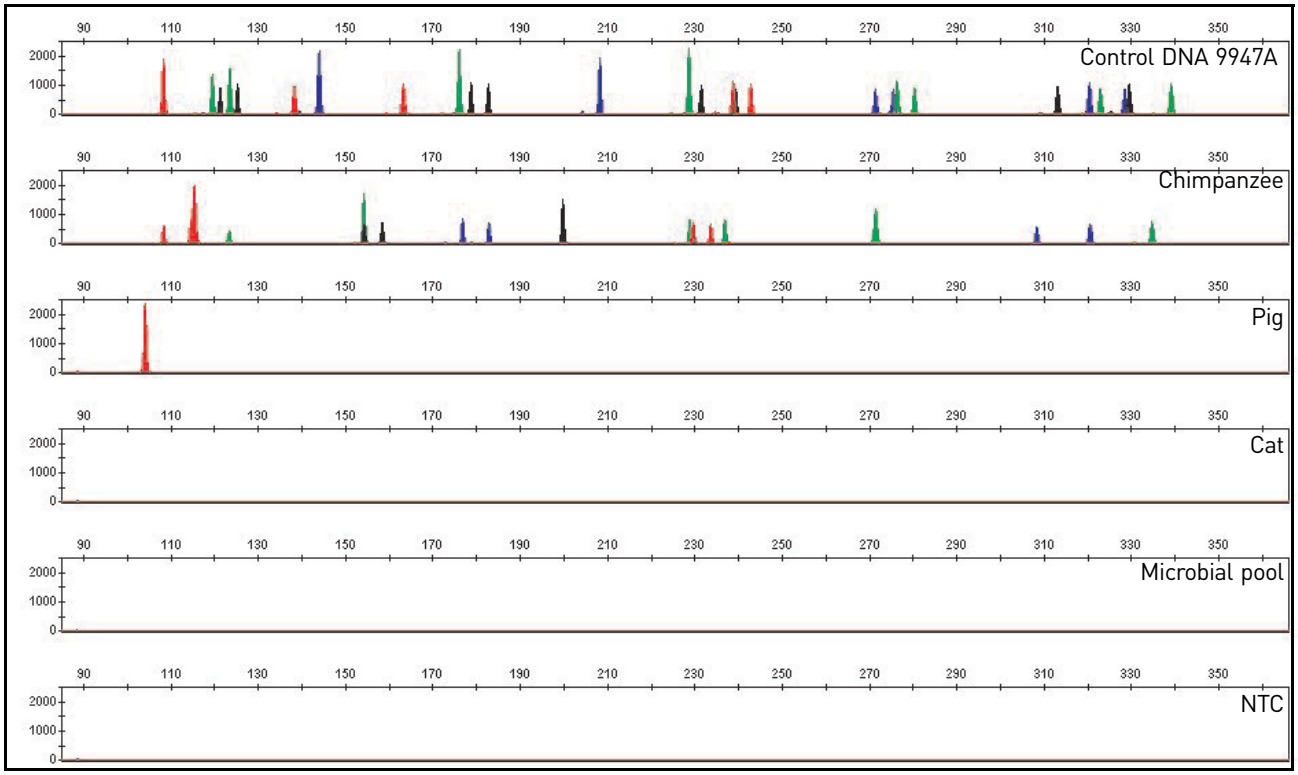


Figure 13 shows amplification for: Control DNA 9947A (1 ng, panel 1), chimpanzee (1 ng, panel 2), pig (10 ng, panel 3), cat (10 ng, panel 4), microbial DNA pool (equivalent to 105 copies of *Candida albicans, Enterococcus faecalis, Escherichia coli, Fusobacterium nucleatum, Lactobacillus casei, Staphylococcus aureus, Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, and Streptococcus viridans*, panel 5), and the non-template control (panel 6). The extracted DNA samples were amplified with the Identifiler® Direct Kit and analyzed using the Applied Biosystems 3130*xl* Genetic Analyzer.

- Primates: gorilla, chimpanzee, orangutan, and macaque (1 ng each)
- Non-primates: mouse, dog, pig, cat, horse, hamster, rat, chicken, and cow (10 ng each)
- Microorganisms: *Candida albicans, Enterococcus faecalis, Escherichia coli, Fusobacterium nucleatum, Lactobacillus casei, Staphylococcus aureus, Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, and Streptococcus viridans* (equivalent to 105 copies). These microorganisms are commonly found in the oral cavity (Suido *et al.*, 1986; Guthmiller *et al.*, 2001).

All the primate DNA samples amplified, producing fragments within the 100 to 350 base pair region (Lazaruk, *et al.*, 2001; Wallin, *et al.*, 1998).

The microorganisms, chicken, cat, hamster, rat, rabbit, and mouse samples did not yield detectable product. Horse, cow, dog, and pig samples produced a 104-bp fragment near the Amelogenin locus in PET® dye.

Sensitivity

SWGDAM guideline 2.3

"When appropriate, the range of DNA quantities able to produce reliable typing results should be determined." (SWGDAM, July 2003)

Blood on FTA® cards

The Identifiler® Direct Kit has been optimized at 25 µL PCR reaction volume to overcome the PCR inhibition expected when amplifying blood samples directly from unpurified 1.2 mm FTA® discs. Depending on the volume of blood spotted onto the FTA® card, DNA quantities present on the 1.2 mm disc may vary from laboratory to laboratory. It is essential for your laboratory to optimize the PCR conditions based on the types of blood samples received or based on your standard operating protocol used in the spotting of blood onto FTA® cards. Refer to page 19 for instructions on PCR optimization.

Buccal cells on FTA® or Indicating FTA® cards and buccal cells on Bode DNA Collectors

The Identifiler® Direct Kit has been optimized at 25 µL PCR reaction volume to overcome the PCR inhibition expected when amplifying buccal cells directly from unpurified 1.2 mm FTA® discs or Indicating FTA® discs. Depending on the collecting devices used, the collection methods applied, and the swab-to-FTA® transfer protocol employed, DNA quantities present on the 1.2 mm disc may vary from sample to sample and from laboratory to laboratory. It is essential for your laboratory to optimize the PCR conditions based on the types of buccal samples received or based on your standard operating protocol used in transferring saliva from a buccal swab onto an FTA® card or Indicating FTA® cards. Refer to page 19 for instructions on PCR optimization.

Effect of DNA quantity on results

If too much DNA is added to the PCR reaction, the increased amount of PCR product that is generated can result in:

- Fluorescence intensity that exceeds the linear dynamic range for detection by the instrument ("off-scale" data).

  Off-scale data is a problem because:

  – Quantitation (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.

  – Multicomponent analysis of off-scale data is not accurate. This inaccuracy results in poor spectral separation ("pull-up").

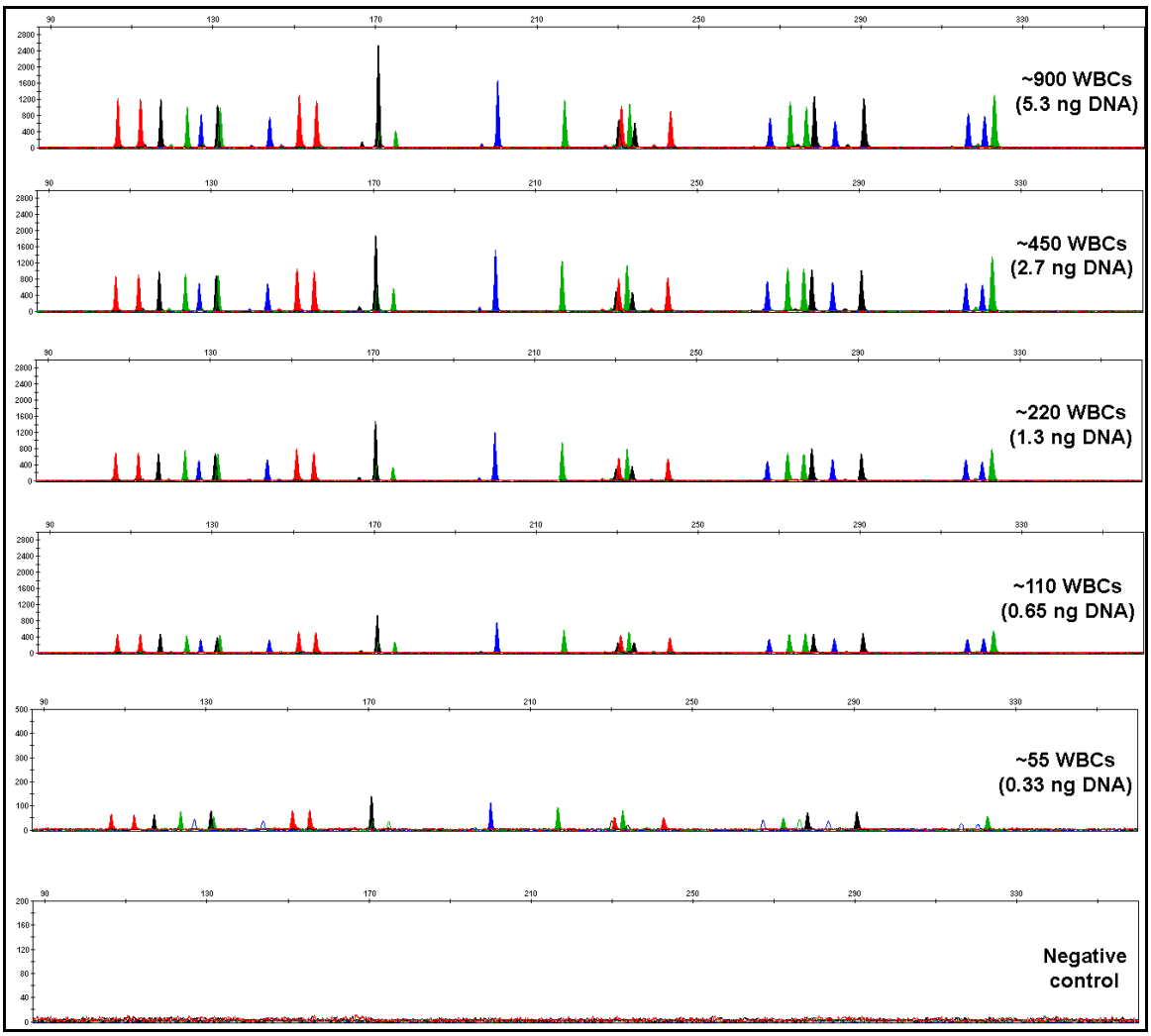
- Incomplete +A nucleotide addition.

  To ensure minimal occurrence of offscale data when using the Identifiler® Direct Kit, optimize PCR cycle number according to instructions on page 19.

When the total number of allele copies added to the PCR is extremely low, unbalanced amplification of the alleles may occur because of stochastic fluctuation.

Individual laboratories may find it useful to determine an appropriate minimum peak height threshold based on their own results and instruments using low amounts of input DNA.

Figure 14 Effect of amplifying varying amounts of white blood cells (WBCs) spotted onto Indicating FTA® discs



Note that the y-axis scale is magnified for the lower amounts of DNA, analyzed using the Applied Biosystems® 3130*xl* Genetic Analyzer. The amount of DNA on the Indicating FTA® cards were calculated based on the assumptions of 100% cell lysis efficiency and that each cell contain 6 pg of DNA.

The results from white blood cells spotted onto Bode DNA Collectors were comparable to the results shown here obtained using the Identifiler® Direct Kit with white blood cells spotted onto FTA® Indicating Cards (data not shown).

Stability

SWGDAM guideline 2.4

"The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults has been extensively documented. In most instances, assessment of the effects of these factors on new forensic DNA procedures is not required. However, if substrates and/or environmental and/or chemical insults could potentially affect the analytical process, then the process should be evaluated using known samples to determine the effects of such factors." (SWGDAM, July 2003)

DNA on FTA® cards

Aged blood on FTA® cards and aged buccal cells on Indicating FTA® cards were prepared to examine the sample-on-substrate stability. Finger-prick blood spotted onto FTA® card and buccal samples swabbed and transferred using the EasiCollect™ devices were collected on three individuals over the course of 30 weeks. The Identifiler® Direct Kit was used to amplify the aged FTA® samples in a GeneAmp® PCR System 9700 with the gold-plated silver 96-well block and were electrophoresed and detected using an Applied Biosystems® 3130*xl* Genetic Analyzer. The results of the aged blood on FTA® card are shown in Figure 15 and the results of the aged buccal cells on Indicating FTA® card are shown in Figure 16. The analysis revealed that the age of the FTA® samples did not impact the performance of the AmpFℓSTR® Identifiler® Direct Kit.

Figure 15 Amplification of blood on FTA® card stored for various amounts of time at room temperature

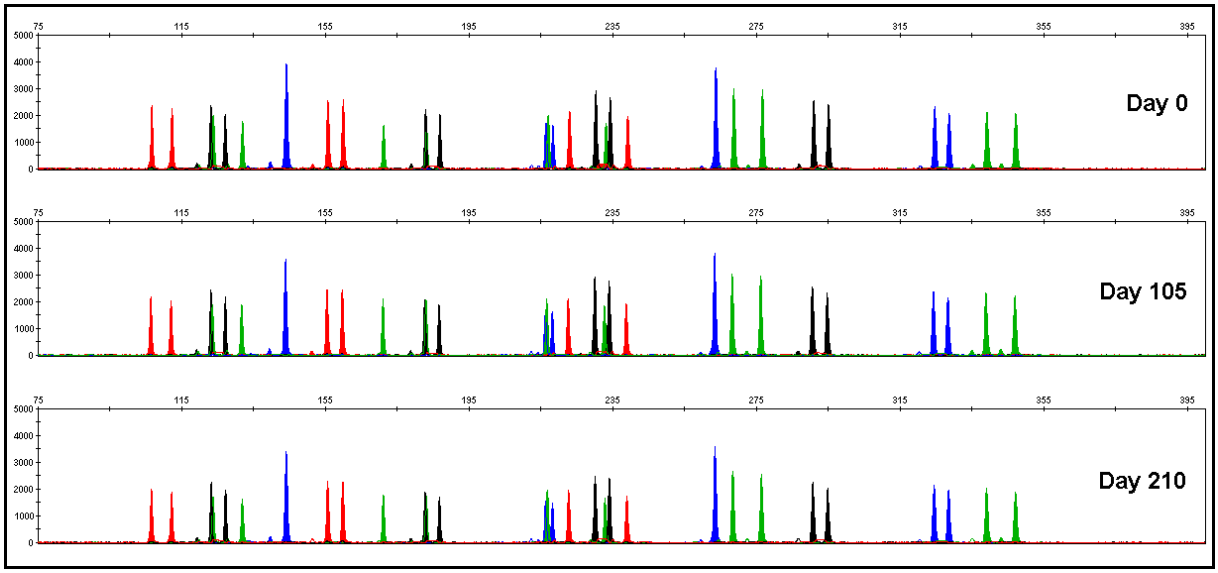
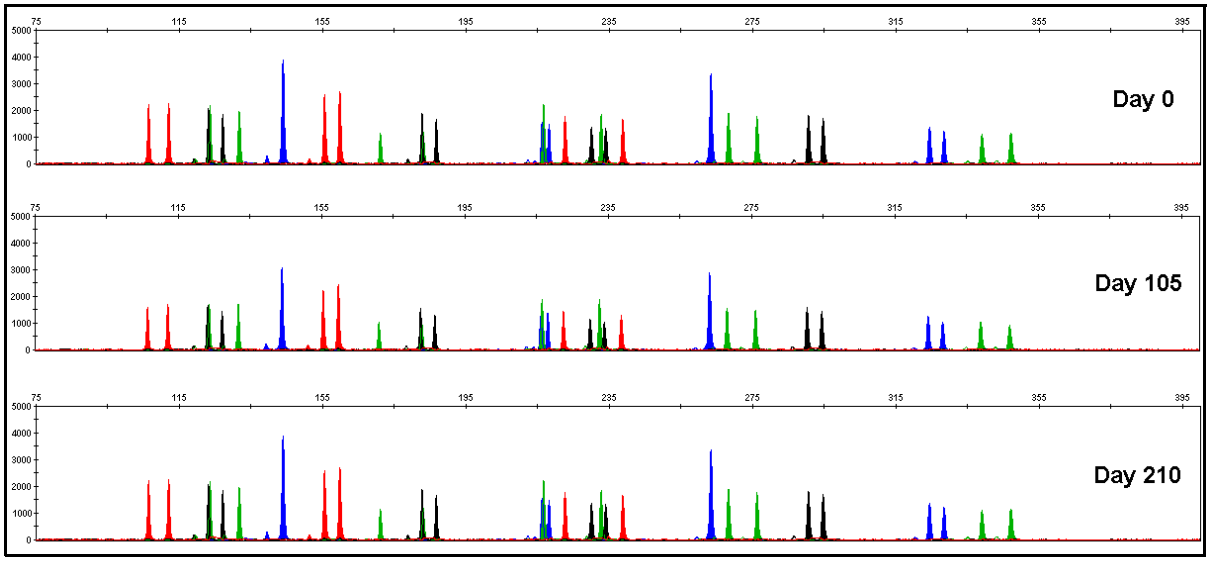


Figure 16 Amplification of buccal cells on Indicating FTA® card stored for various amounts of time at room temperature



DNA on buccal swabs

Aged buccal cell samples on Copan 4N6 FLOQSwabs®, Whatman OmniSwabs, and Puritan swabs were also prepared to verify their respective sample-on-substrate stability. Buccal swabs were collected from 40 individuals on each swab type over the course of three months. The aged swab samples were processed with Prep-n-Go™ Buffer, amplified using the Identifiler® Direct Kit in a GeneAmp® PCR System 9700 with the gold-plated silver 96-well block, and were electrophoresed and detected using an Applied Biosystems® 3130*xl* Genetic Analyzer. Figure 17 shows the results of the aged buccal samples collected on each swab type and lysed at 90°C for 20 minutes. For comparison, Figure 18 shows the results of fresh buccal samples collected on Copan 4N6 FLOQSwabs® and lysed at room temperature.

The analysis revealed that buccal samples on the swab types tested, air-dried immediately after collection, and aged up to three months at room temperature produce acceptable profiles when amplified with the Identifiler® Direct Kit.

Figure 17 Amplification of buccal cells on aged Copan 4N6 FLOQSwabs®, OmniSwabs, and Puritan swabs stored for 3 months at room temperature and lysed with Prep-n-Go™ Buffer at 99°C for 20 minutes

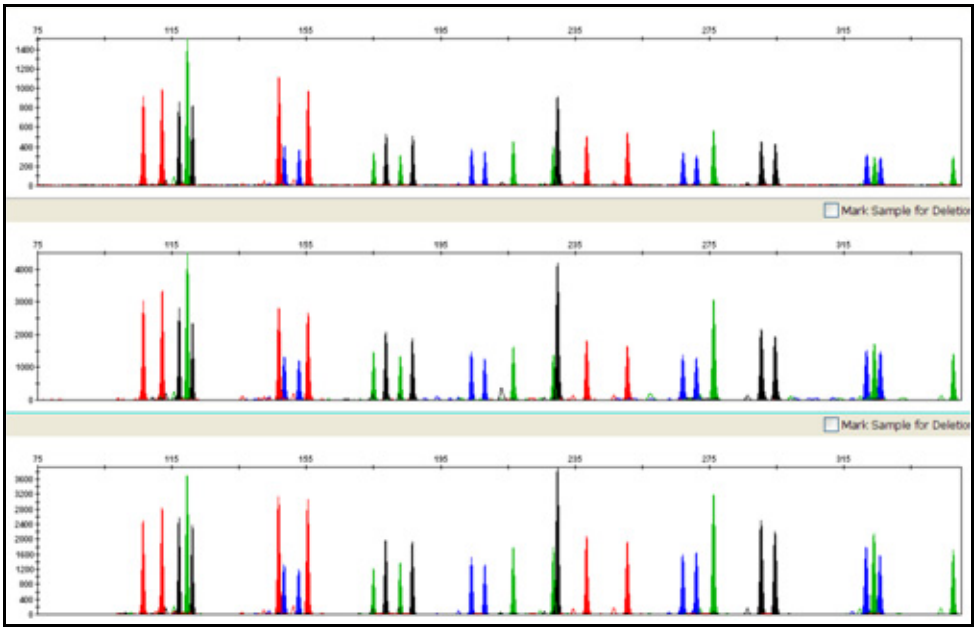
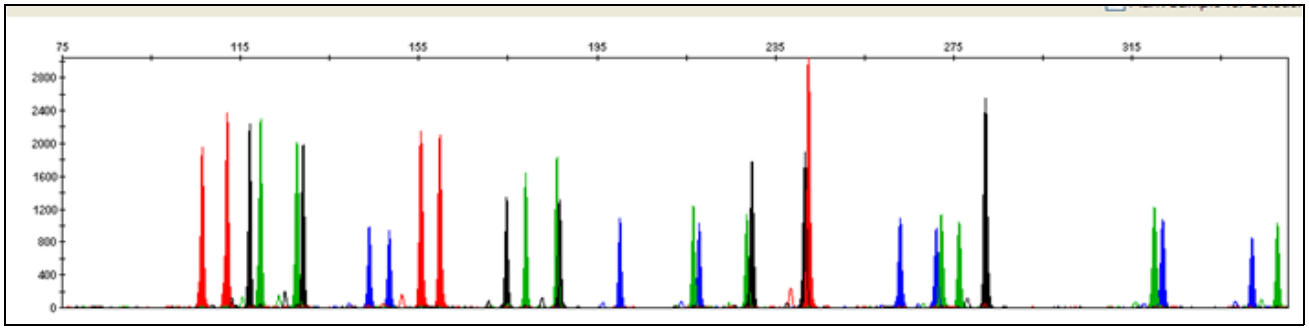


Figure 18 Amplification of buccal cells on fresh Copan 4N6 FLOQSwabs® and lysed with Prep-n-Go™ Buffer at room temperature



Population data

SWGDAM guideline 2.7

"The distribution of genetic markers in populations should be determined in relevant population groups." (SWGDAM, July 2003)

Overview

To interpret the significance of a match between genetically typed samples, you must know the population distribution of alleles at each locus in question. If the genotype of the relevant evidence sample is different from the genotype of a suspects reference sample, then the suspect is excluded as the donor of the biological evidence that was tested. An exclusion is independent of the frequency of the two genotypes in the population.

If the suspect and evidence samples have the same genotype, then the suspect is included as a possible source of the evidence sample. The probability that another, unrelated individual would also match the evidence sample is estimated by the frequency of that genotype in the relevant population(s).

Population samples used in these studies

The Identifiler® Kit, prior to the addition of the D8S1179 degenerate primer, was used to generate the population data provided in this section. Samples were collected from individuals throughout the United States with no geographical preference.

Population	Number of samples	Samples provided by
African-American	357	Kentucky State Police and the Federal Bureau of Investigation
U.S. Caucasian	349	
U.S. Hispanic	290	Minnesota Bureau of Criminal Apprehension/Memorial Blood Center of Minneapolis
Native American	191	

In addition to the alleles that were observed and recorded in the Life Technologies databases, other alleles have been published or reported to Life Technologies by other laboratories (see the STRBase at **www.cstl.nist.gov/div831/strbase**).

Identifiler® Direct Kit allele frequencies

Table 5 shows the Identifiler® Direct Kit allele frequencies in four populations, listed as percentages.

Table 5 Identifiler® Direct Kit allele frequencies

Allele	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
CSF1PO				
6	†	†	†	†
7	4.62	0.14†	0.34†	†
8	7.56	0.29†	0.17†	0.52†
9	3.78	1.72	0.86†	8.38
10	27.87	24.21	23.10	30.89
11	20.59	31.91	28.28	21.99

Allele	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
11.3	0.14†	†	†	†
12	29.13	32.81	39.66	32.72
13	5.32	7.31	6.38	4.71
14	0.98	1.43	0.86†	0.79†
15	†	0.29†	†	†
D2S1338				
15	0.14†	†	†	†
16	5.32	4.73	2.41	2.62
17	10.78	17.34	21.21	9.95
18	5.60	6.30	4.14	7.07
19	14.15	13.75	22.76	29.58
20	6.02	14.61	13.79	9.69
21	14.01	2.58	2.59	2.38
22	13.17	4.01	7.41	15.18
23	10.78	11.46	11.36	11.78
24	9.80	11.75	8.45	7.85
25	8.12	10.60	5.17	3.14
26	1.96	2.72	0.69†	0.79†
27	0.14†	0.14†	†	†
28	†	†	†	†
D3S1358				
<11	0.42†	0.14†	†	†
11	†	†	†	0.26†
12	0.56†	†	0.17†	†
13	0.70†	0.29†	0.17†	†
14	12.04	15.76	7.41	6.81
15	30.53	25.36	39.14	40.84
15.2	0.14†	†	†	†
16	28.57	22.78	26.72	32.98
17	19.47	18.19	16.03	9.95
18	6.72	16.48	8.97	8.38
19	0.84	1.00	1.03	0.79†
20	†	†	0.34†	†

Allele	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
D5S818				
7	0.14†	†	6.72	15.71
8	5.46	†	0.69†	†
9	1.68	4.15	5.17	6.02
10	6.72	5.44	5.17	4.19
11	25.49	39.26	39.14	41.10
12	36.41	35.24	29.31	23.30
13	21.57	15.47	12.59	9.42
14	2.38	0.14†	0.69†	0.26†
15	†	0.29†	0.18†	†
16	†	†	0.17†	†
17	0.14†	†	0.17†	†
D7S820				
6	†	0.14†	0.17†	†
7	0.42†	1.29	1.72	0.52†
8	18.77	16.48	11.72	13.09
9	13.73	17.62	6.21	8.12
10	34.45	27.22	27.41	21.99
11	19.89	18.05	28.79	28.80
12	10.78	14.76	20.17	24.08
13	1.54	3.72	3.45	3.40
14	0.42†	0.72	0.34†	†
15	†	†	†	†
D8S1179				
8	0.42†	2.29	0.34†	0.52†
9	0.42†	1.15	0.34†	0.26†
10	2.38	9.74	8.45	4.71
11	3.92	6.02	5.86	3.40
12	13.31	14.04	12.07	11.52
13	23.25	32.52	32.93	37.43
14	30.11	21.35	26.21	30.63
15	20.17	9.89	10.86	9.42
16	4.62	2.72	2.41	1.57
17	1.12	0.29†	0.52†	0.52†
18	0.28†	†	†	†
19	†	†	†	†

Allele	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
D13S317				
8	3.08	12.18	9.66	4.97
9	2.52	7.74	21.72	17.80
10	3.78	4.44	9.14	13.61
11	24.51	29.80	23.10	24.35
12	46.22	30.80	20.86	23.04
13	15.41	11.17	10.17	7.85
14	4.34	3.72	5.34	8.12
15	0.14†	0.14†	†	0.26†
D16S539				
5	†	†	†	†
8	3.22	1.72	1.72	0.79†
9	19.05	10.46	9.31	12.30
10	10.92	5.59	15.69	15.45
11	31.51	31.95	30.17	30.89
12	18.77	30.23	29.48	27.75
13	14.85	16.76	11.55	10.73
14	1.54	3.01	2.07	2.09
15	0.14†	0.29†	†	†
D18S51				
7	†	†	†	†
9	0.14†	†	†	†
10	0.28†	0.86	0.52†	0.79†
10.2	0.14†	†	†	†
11	0.28†	1.15	1.21	†
12	7.00	13.90	10.34	14.92
13	4.34	12.18	14.48	9.16
13.2	0.42†	†	†	†
14	6.86	16.76	15.52	26.96
14.2	0.28†	†	†	†
15	19.47	13.61	16.55	12.04
16	16.53	13.61	11.72	10.73
17	18.21	12.32	14.14	14.66
18	11.90	7.74	6.72	2.62
19	6.02	4.44	4.14	3.93
20	4.90	1.72	2.24	1.83

Allele	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
21	2.10	1.00	1.03	1.31
22	0.70†	0.43†	0.52†	0.79†
23	0.42†	0.14†	0.52†	0.26†
24	†	0.14†	0.17†	†
25	†	†	0.17†	†
26	†	†	†	†
27	†	†	†	†
D19S433				
9	†	0.14†	0.17†	†
10	1.54	†	†	†
11	7.14	0.72	0.52†	0.52†
11.2	0.14†	†	0.17†	†
12	10.78	7.74	6.21	3.14
12.2	6.30	0.57†	1.90	†
13	29.83	28.94	16.03	17.80
14	21.01	34.10	31.72	24.87
14.2	4.20	0.86	5.00	3.66
15	4.76	15.76	13.45	13.35
15.2	3.36	2.72	8.79	10.73
16	2.38	4.15	4.31	3.93
16.2	2.38	1.72	2.93	1.83
17	†	0.29†	0.17†	0.79†
17.2	0.28†	0.29†	†	2.88
18.2	0.14†	0.29†	†	1.05†
D21S11				
24	†	†	†	†
24.2	0.14†	0.43†	0.17†	†
24.3	†	†	†	†
25	†	†	†	†
25.2	†	0.14†	0.17†	†
26	0.14†	0.14†	0.17†	†
27	5.04	4.58	1.21	0.52†
28	22.97	16.76	9.14	6.28
28.2	†	†	†	†
29	19.33	20.49	21.21	16.75
29.2	0.14†	†	0.52†	0.26†

Allele	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
29.3	0.14†	†	†	†
30	17.23	25.21	29.31	34.29
30.2	1.40	3.30	2.93	1.83
31	7.98	7.16	6.72	5.76
31.2	7.98	9.46	8.62	18.85
32	1.12	1.43	1.55	0.79†
32.2	5.88	7.16	12.93	9.69
33	0.56†	†	†	0.52†
33.2	3.78	3.30	4.14	3.66
34	1.26	†	†	†
34.1	0.14†	†	†	†
34.2	0.14†	0.29†	0.86†	0.79†
35	2.94	†	0.34†	†
35.1	0.14†	†	†	†
35.2	†	0.14†	†	†
36	0.84	†	†	†
37	0.28†	†	†	†
38	0.14†	†	†	†
FGA				
16	†	0.14†	†	†
16.1	0.14†	†	†	†
17	†	0.29†	0.17†	†
17.2	0.14†	†	†	†
18	0.70†	2.72	0.52†	1.31
18.2	1.40	†	†	†
19	6.72	6.16	7.07	10.21
19.2	0.28†	†	†	†
20	7.00	13.90	7.41	12.30
20.2	†	0.14†	†	†
21	12.89	16.91	14.66	12.83
22	21.57	16.91	17.24	10.47
22.2	0.28†	1.29	0.34†	0.26†
22.3	0.14†	0.14†	†	†
23	14.99	15.19	11.90	15.97
23.2	0.14†	0.14†	0.86†	0.26†
24	17.51	13.75	15.34	15.71

Allele	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
24.2	†	0.14†	0.17†	†
25	7.98	8.60	14.14	14.14
26	3.50	2.72	6.90	4.45
26.2	†	†	†	0.52
29	0.56†	†	†	†
30	†	†	†	†
30.2	0.14†	†	†	†
31.2	†	†	†	†
32.2	†	†	†	†
33.2	†	†	†	†
34.2	0.14†	†	†	†
42.2	†	†	†	†
43.2	†	†	†	†
44.2	0.28†	†	†	†
45.2	†	†	†	0.26†
46.2	0.14†	†	†	†
47.2	†	†	†	†
48.2	0.14†	†	†	†
50.2	†	†	†	†
51.2	†	†	†	†
TH01				
4	†	†	†	†
5	0.28†	0.43†	0.17†	†
6	11.06	20.49	22.76	20.68
7	42.86	21.78	33.62	43.98
8	20.73	11.46	8.45	5.24
8.3	†	†	†	†
9	†	†	†	6.28
9.3	11.62	29.08	20.34	23.56
10	0.98	0.43†	0.52†	0.26†
11	†	†	†	†
13.3	0.14†	†	†	†
TPOX				
6	6.72	0.14†	0.34†	†
7	2.24	†	0.34†	0.26†
8	36.13	53.30	49.66	37.96

Allele	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
9	21.15	11.60	7.24	4.19
10	9.24	4.30	4.66	3.40
11	21.43	25.93	27.24	39.27
12	3.08	4.73	10.52	14.92
13	†	†	†	†
vWA				
11	0.28†	†	0.17†	†
12	†	†	†	0.26†
13	1.26	0.43†	†	0.26†
14	7.14	8.31	6.90	4.45
15	20.03	11.32	10.00	7.07
16	26.75	23.35	34.31	32.98
17	20.59	24.50	21.55	33.51
18	14.71	22.49	18.45	15.45
19	6.72	8.31	7.07	4.71
20	1.96	1.15	1.38	1.05†
21	0.28†	†	0.17†	0.26†
22	0.28†	†	†	†
23	†	†	†	†
24	†	0.14†	†	†

† A minimum allele frequency (0.7% for the African-American database, 0.7% for the U.S. Caucasian database, 0.9% for the U.S. Hispanic database, and 1.3% for the Native American database) is suggested by the National Research Council in forensic calculations.

Low-frequency alleles

Some alleles of the Identifiler® Direct Kit loci occur at a low frequency. For these alleles, a minimum frequency (5 divided by 2n, where n equals the number of individuals in the database) was assigned for the Identifiler® Direct Kit African-American, Asian, U.S. Caucasian, and U.S. Hispanic databases, as suggested in the 1996 report of the Committee on DNA Forensic Science (National Research Council, 1996). These databases are summarized in Table 8 on page 101. The minimum reportable genotype frequency at each locus is: 1.19×10^{-4} for the African-American database; 1.19×10^{-4} for the U.S. Caucasian database; 1.70×10^{-4} for the U.S. Hispanic database; and 2.97×10^{-4} for the Native American database [$p2 + p(1–p)\ \theta$, where $\theta = 0.01$]. Hence, the minimum combined multilocus genotype frequency at 15 loci is: 1.36×10^{-59} for the African-American database; 1.36×10^{-59} for the U.S. Caucasian database; 2.86×10^{-57} for the U.S. Hispanic database; and 1.23×10^{-53} for the Native American database.

Evaluation of Hardy-Weinberg equilibrium

Estimates of expected heterozygosity (HExp) were computed as described by Nei, M. (1973) using the program PopGene 1.32. Possible divergence from Hardy-Weinberg expectations (HWE) was tested using various methods: by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (Levene, H., Nei, M. 1978) and using chi-square (HW X^2p) and likelihood ratio (HW G^2p) tests (as implemented in the program PopGene 1.32): and with an exact test (HW Exact p), which is a Markov chain method, based on 1000 shuffling experiments, to estimate without bias the exact P-value of the Hardy-Weinberg test with multiple alleles (Guo, S.W. 1992), as implemented in the program GenePop 3.4. An inter-class correlation test analysis (Burrows' composite measure of linkage disequilibria between pairs of loci and X^2 tests for significance [Weir, B. 1990]) was performed separately in each population to detect any correlations between alleles at any of the pair-wise comparisons of the 15 loci, using the program PopGene 1.32.

Observed heterozygosity (H_o), expected heterozygosity, information content, and tests for detecting departures from Hardy-Weinberg equilibrium are shown for each population in Table 6. While a number of the chi-square tests gave seemingly significant p-values (putatively indicating departures from Hardy-Weinberg equilibrium), chi-squared tests are very sensitive to small expected values (as in the case of multiple rare alleles where the expected number of certain genotypes is 1 or fewer, such as with some of these markers), and can greatly inflate the test statistic in this situation (Weir, B. 1990). With the exact test, the number of tests with p-value < 0.05 were 0 in the African American and U.S. Caucasian populations, 1 in the U.S. Hispanic population (D8S1179; p=0.0304) and 2 in the Native Americans (D21S11, p=0.0118; D5S818, p=0.0205). These are no more than would be expected by chance. No more alleles were observed to be in linkage disequilibrium than would be expected by chance alone. The average observed heterozygosity across the 15 STR loci was 0.804 in the African American population, 0.792 in the U.S. Caucasian sample population, 0.793 in the Hispanic sample population, and 0.757 in the Native Americans. The most heterozygous locus was FGA (mean observed heterozygosity across all populations of 0.875), and the least heterozygous STR locus was TPOX (mean observed heterozygosity across all populations of 0.677).

Table 6 Heterozygosity and p-values for Hardy-Weinberg tests of the 15 Identifiler STR loci in four U.S. populations†

	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
CSF1P0				
HW X^2 p	0.13649	0.926431	0.951476	0.839278
HW G^2 p	0.08902	0.894972	0.918038	0.728023
HW Exact p	0.0762	0.2688	0.5456	0.6148
CSF1P0 *(continued)*				
HExp	0.7829	0.7267	0.7051	0.7398
H_o	0.7703	0.7421	0.7138	0.7958
D2S1338				

† HW X^2 p, probability value of X^2 test for Hardy-Weinberg equilibrium; HW G^2 p, probability value of the G-statistic of the Likelihood Ratio test for multinomial proportions; HW Exact p; A Markov chain unbiased exact test to estimate the P-value of the Hardy-Weinberg test with multiple alleles; Hexp, Expected heterozygosity; H_o, observed heterozygosity

	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
HW X^2 p	0.409878	0.537758	0.975972	0.722543
HW G^2 p	0.962501	0.407932	0.973054	0.760953
HW Exact p	0.7838	0.3488	0.9794	0.5825
HExp	0.8936	0.8823	0.8529	0.8428
H_o	0.8768	0.8653	0.8379	0.801
D3S1358				
HW X^2 p	0.947371	0.670787	0.681659	0.087223
HW G^2 p	0.907905	0.654776	0.852278	0.175807
HW Exact p	0.2967	0.2814	0.4684	0.0614
HExp	0.7681	0.7986	0.7361	0.7028
H_o	0.7955	0.8166	0.7414	0.7382
D5S818				
HW X^2 p	0.993751	0.859805	0.944725	0.073002
HW G^2 p	0.989776	0.520417	0.979044	0.08025
HW Exact p	0.958	0.462	0.4662	0.0205
HExp	0.7476	0.6931	0.7351	0.7378
H_o	0.7479	0.7077	0.7586	0.6806
D7S820				
HW X^2 p	0.987668	0.571989	0.336834	0.324754
HW G^2 p	0.969887	0.44694	0.687948	0.289733
HW Exact p	0.9818	0.2286	0.4028	0.1276
HExp	0.7758	0.8117	0.7822	0.7858
H_o	0.7955	0.7908	0.7862	0.7487
D8S1179				
HW X^2 p	0.067164	0.545414	0.047783	0.446248
HW G^2 p	0.568837	0.275218	0.302937	0.760077
HW Exact p	0.2176	0.3264	0.0304	0.1656
HExp	0.7925	0.8047	0.7853	0.7403
H_o	0.7899	0.8424	0.8	0.6806

	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
D13S317				
HW X^2 p	0.014379	0.711127	0.353995	0.813948
HW G^2 p	0.609389	0.871173	0.190736	0.814681
HW Exact p	0.3818	0.667	0.2415	0.6851
HExp	0.6977	0.7797	0.8251	0.8222
H_o	0.6695	0.7364	0.8207	0.8168
D16S539				
HW X^2 p	0.433216	0.67702	0.058631	0.996396
HW G^2 p	0.482435	0.594871	0.37601	0.981384
HW Exact p	0.3753	0.4328	0.3068	0.9986
HExp	0.7939	0.7632	0.7747	0.7766
H_o	0.8263	0.7822	0.7828	0.7853
D18S51				
HW X^2 p	0.999844	0.628334	0.999203	0.343027
HW G^2 p	1	0.872113	0.999492	0.798859
HW Exact p	0.978	0.0982	0.9152	0.2265
HExp	0.8694	0.8769	0.8761	0.8463
H_o	0.8824	0.8682	0.8862	0.8377
D19S433				
HW X^2 p	0.91703	0.806717	0.731222	0.810711
HW G^2 p	0.83419	0.999765	0.975476	0.898389
HW Exact p	0.4517	0.69	0.3475	0.4301
HExp	0.8364	0.7659	0.8310	0.8430
H_o	0.8011	0.7622	0.8414	0.822
D21S11				
HW X^2 p	0.985687	0.936146	0	0
HW G^2 p	1	0.999757	0.999794	0.712937
HW Exact p	0.7627	0.7861	0.6476	0.0118
HExp	0.8585	0.8427	0.8290	0.8003
H_o	0.8711	0.8567	0.7931	0.801
FGA				
HW X^2 p	0	0.904953	0.263223	0.999686
HW G^2 p	1	0.999812	0.960137	0.999946
HW Exact p	0.9761	0.4459	0.0891	0.9161
HExp	0.8659	0.8686	0.8751	0.8746
H_o	0.8824	0.8854	0.8724	0.8482

	African-American (n = 357)	U.S. Caucasian (n = 349)	U.S. Hispanic (n = 290)	Native American (n = 191)
TH01				
HW X^2 p	0.961911	0.997905	0.649467	0.329461
HW G^2 p	0.940414	0.99169	0.617212	0.318591
HW Exact p	0.8286	0.9716	0.4495	0.1377
HExp	0.7323	0.7866	0.7666	0.7016
H_o	0.7395	0.7822	0.8103	0.6492
TPOX				
HW X^2 p	0.765163	0.801518	0.875348	0.333914
HW G^2 p	0.611014	0.757735	0.913091	0.229017
HW Exact p	0.7247	0.5775	0.8356	0.0647
HExp	0.7643	0.6311	0.6607	0.6765
H_o	0.7563	0.6304	0.6759	0.6178
vWA				
HW X^2 p	0.925176	0.005048	0.641684	0.994248
HW G^2 p	0.964308	0.218817	0.934427	0.997184
HW Exact p	0.7033	0.0564	0.7066	0.8845
HExp	0.8141	0.8081	0.7818	0.7457
H_o	0.8571	0.8138	0.7759	0.7277

Concordance studies

We compared allele calls between the Identifiler® and Identifiler® Direct Kits.

The genotype data from the 200 analyzed treated paper workflow samples showed 100% concordance between the Identifiler® and Identifiler® Direct Kits.

The genotype data from 84 buccal samples processed using Prep-n-Go™ Buffer and the Identifiler® Direct Kit showed 100% concordance to allele calls generated for purified DNA samples analyzed with the Identifiler® kit.

Mutation rate

Estimation of spontaneous or induced germline mutation at genetic loci can be achieved by comparing the genotypes of offspring to those of their parents. From such comparisons the number of observed mutations are counted directly.

In previous studies, genotypes of ten STR loci that were amplified by the AmpFℓSTR® SGM Plus® PCR Amplification Kit were determined for a total of 146 parent-offspring allelic transfers (meioses) at the Forensic Science Service, Birmingham, England. One length-based STR mutation was observed at the D18S11 locus; mutations were not detected at any of the other nine STR loci. The D18S11 mutation was represented by an increase of one 4-nt repeat unit, allele 17 was inherited as allele 18 (single-step mutation). The maternal/paternal source of this mutation could not be distinguished.

Additional mutation studies

Additional studies (Edwards *et al.,* 1991; Edwards *et al.,* 1992; Weber and Wong, 1993; Hammond *et al.,* 1994; Brinkmann *et al.,* 1995; Chakraborty *et al.,* 1996; Chakraborty *et al.,* 1997; Brinkmann *et al.,* 1998; Momhinweg *et al.,* 1998; Szibor *et al.,* 1998) of direct mutation rate counts produced:

- Larger sample sizes for some of the Identifiler® Direct Kit loci.
- Methods for modifications of these mutation rates (to infer mutation rates indirectly for those loci where the rates are not large enough to be measured directly and/or to account for those events undetectable as Mendelian errors).

Probability of identity

Table 7 shows the Probability of Identity (P_I) values of the Identifiler® Direct Kit loci individually and combined.

Table 7 Probability of Identity values for the Identifiler® Direct Kit STR loci

Locus	African-American	U.S. Caucasian	U.S. Hispanic	Native American
CSF1PO	0.079	0.132	0.141	0.123
D2S1338	0.023	0.027	0.038	0.043
D3S1358	0.097	0.076	0.112	0.158
D5S818	0.104	0.147	0.115	0.110
D7S820	0.085	0.063	0.083	0.081
D8S1179	0.074	0.064	0.089	0.104
D13S317	0.132	0.079	0.056	0.056
D16S539	0.077	0.097	0.090	0.082
D18S51	0.033	0.031	0.031	0.046
D19S433	0.042	0.087	0.049	0.044
D21S11	0.037	0.044	0.047	0.074
FGA	0.034	0.035	0.032	0.031
TH01	0.109	0.079	0.097	0.134
TPOX	0.089	0.188	0.168	0.159
vWA	0.066	0.066	0.080	0.103
Combined	1.31×10^{-18}	5.01×10^{-18}	7.65×10^{-18}	3.62×10^{-17}

The P_I value is the probability that two individuals selected at random will have an identical Identifiler® Direct Kit genotype (Sensabaugh, 1982). The P_I values for the populations described in this section are then approximately $1/7.64 \times 10^{17}$ (African-American), $1/2.00 \times 10^{17}$ (U.S. Caucasian), $1/1.31 \times 10^{17}$ (U.S. Hispanic), and $1/2.76 \times 10^{16}$ (Native American).

Probability of paternity exclusion

Table 8 shows the Probability of Paternity Exclusion (P_E) values of the Identifiler® Direct Kit STR loci individually and combined.

Table 8 Probability of Paternity Exclusion values for the Identifiler® Direct Kit loci

Locus	African-American	U.S. Caucasian	U.S. Hispanic	Native American
CSF1PO	0.545	0.496	0.450	0.409
D2S1338	0.748	0.725	0.671	0.399
D3S1358	0.591	0.630	0.495	0.510
D5S818	0.506	0.440	0.525	0.601
D7S820	0.591	0.582	0.574	0.492
D8S1179	0.580	0.680	0.599	0.601
D13S317	0.383	0.487	0.638	0.370
D16S539	0.649	0.566	0.567	0.428
D18S51	0.760	0.731	0.767	0.329
D19S433	0.601	0.531	0.678	0.360
D21S11	0.737	0.708	0.586	0.399
FGA	0.760	0.766	0.739	0.309
TH01	0.492	0.566	0.618	0.646
TPOX	0.521	0.329	0.392	0.687
vWA	0.709	0.625	0.555	0.528
Combined	0.9999996	0.9999992	0.9999990	0.9999527

The P_E value is the probability, averaged over all possible mother-child pairs, that a random alleged father will be excluded from paternity after DNA typing of the Identifiler® Direct Kit STR loci (Chakraborty and Stivers, 1996).

A Troubleshooting

Follow the actions recommended in this appendix to troubleshoot problems that occur during analysis.

Table 9 Troubleshooting

Observation	Possible causes	Recommended actions
Faint or no signal from both the AmpFℓSTR® Identifiler® Direct Control DNA 9947A and the DNA test samples at all loci	Incorrect volume or absence of Identifiler® Direct Master Mix or Identifiler® Direct Primer Set	Repeat amplification.
	No activation of AmpliTaq Gold® DNA Polymerase	Repeat amplification, making sure to hold reactions initially at 95°C for 11 minutes.
	Master Mix not vortexed thoroughly before aliquoting	Vortex the Master Mix thoroughly.
	Identifiler® Direct Primer Set exposed to too much light	Store the Primer Set protected from light.
	PCR System malfunction	Refer to the thermal cycler user's manual and check instrument calibration.
	Use of incorrect thermal cycling parameters	Check the protocol for correct thermal cycling parameters.
	MicroAmp® Base used with tray/ retainer set and tubes in GeneAmp® 9700	Remove MicroAmp Base from tray/retainer set and repeat test.
	Insufficient PCR product electrokinetically injected	Prepare PCR product as described in Chapter 3, "Perform Electrophoresis" on page 29.
	Degraded formamide	Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.
	Sample punch location was not optimal	For blood samples on treated paper, punch in the center of the blood stain. For buccal samples on treated paper, punch in the center of the buccal transfer or punch in the optimal spot based on past experiences. For buccal samples collected with the Bode Buccal DNA Collector™, punch from near the tip of the collector.
	Insufficient lysis of the swab head	Ensure swab heads are incubated for 20 minutes in 400 µL Prep-N-Go™ Buffer.

Observation	Possible causes	Recommended actions
More than two alleles present at a locus	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Amplification of stutter product (n-4 nt position)	See "Stutter products" on page 75.
	Incomplete 3´ A base addition (n-1 nt position)	See "Addition of 3´ A nucleotide" on page 80. Be sure to include the final extension step of 60°C for 10 minutes in the PCR.
	Signal exceeds dynamic range of instrument (off-scale data)	Ensure cycle number is optimized according to instructions on page 19. Repeat PCR amplification using fewer PCR cycles or use your laboratory's SOP to analyze off-scale data.
	Poor spectral separation (bad matrix)	Follow the steps for creating a spectral file.
		Confirm that Filter Set G5 modules are installed and used for analysis.
	Contamination carried over from the disc punching tool	Clean the disc punching tool thoroughly. If necessary, include a blank punch step in between the sample punches.
	Incomplete denaturation of double stranded DNA	Use recommended amount of Hi-Di™ Formamide and perform heat denaturation step according to the instructions in Chapter 3, "Perform Electrophoresis".
Some but not all loci visible on electropherogram of DNA Test Samples	Disc size used in the amplification reaction was greater than 1.2 mm	Repeat amplification using a use 1.2 mm punch size.
	Insufficient volume of swab lysate added to the reaction	Repeat amplification using the recommended lysate input volume.
	Less than 25 µL of PCR reaction volume was used	Repeat amplification using the recommended PCR reaction volume of 25 µL.
STR profiles contain many off-scale alleles	PCR cycle number was too high	Perform sensitivity experiment (page 19) to determine the optimal PCR cycle number based on the sample type.
	For blood samples: Too much liquid blood was spotted onto paper substrate	Spot <100 µL of liquid blood per sample area.

B Ordering Information

Equipment and materials not included

Table 10 and Table 11 list required and optional equipment and materials not supplied with the Identifiler® Direct Kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

Table 10 Equipment

Equipment	Source
3100/3100-*Avant* Genetic Analyzer	Contact your local Life Technologies sales representative
Applied Biosystems® 3500/3500xL Genetic Analyzer	
Applied Biosystems® 3130/3130*xl* Genetic Analyzer	
Applied Biosystems® 3730 Genetic Analyzer	
GeneAmp® PCR System 9700 with the Silver 96-Well Block	N8050001
GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block	4314878
Silver 96-Well Sample Block	N8050251
Gold-plated Silver 96-Well Sample Block	4314443
Veriti® 96-Well Thermal Cycler	4375786
Tabletop centrifuge with 96-Well Plate Adapters (optional)	MLS
Harris Manual Punch, 1.2 mm	MLS
BSD600-Duet Semi-Automated Dried Sample Punch Instrument with a 1.2 mm punch head	Contact your local Life Technologies support representative for information.
BSD1000-GenePunch Automated Dried Sample Punch Instrument with a 1.2 mm punch head	
Bode Buccal DNA Collector™	4467893 This part number is not available for sale in the US.
Copan FLOQSwabs®	Contact your local Life Technologies support representative for information.
Copan NUCLEIC-CARD™ system	Contact your local Life Technologies support representative for information. This product is not available for sale in the US.

Equipment	Source
96-well, deep-well plate	4392904

Table 11 User-supplied materials

Item†	Source
AmpF*l*STR® Identifiler® Direct PCR Amplification Kit, 200 reaction	4467831
AmpF*l*STR® Identifiler® Direct PCR Amplification Kit, 1000 reaction	4408580
Prep-n-Go™ Buffer (untreated paper substrate)	4467079
Prep-n-Go™ Buffer (buccal swab)	4471406
3100 Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130*xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4® Polymer for 3100/3100-*Avant* Genetic Analyzers	4316355
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 500 LIZ® Size Standard	4322682
OR	*OR*
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10X	402824
Hi-Di™ Formamide	4311320
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-Well Reaction Plate	N8010560
250-µL Glass Syringe (array-fill syringe)	4304470
5.0-mL Glass Syringe (polymer-reserve syringe)	628-3731
For a complete list of parts and accessories for the 3100 instrument, refer to Appendix B of the *3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (Part no. 4335393).	
3130*xl* Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130*xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4® Polymer for 3130/3130*xl* Genetic Analyzers	4352755
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 500 LIZ® Size Standard	4322682
OR	*OR*
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10X	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-Well Reaction Plate	N8010560

Item†	Source
Hi-Di™ Formamide	4311320
For a complete list of parts and accessories for the 3130*xl* instrument, refer to Appendix A of the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (Part no. 4352716).	
3500/3500xL Analyzer materials	
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
POP-4® polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4® polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715
For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide* (PN 4401661).	
3730 Analyzer materials	
3730 DNA Analyzer Capillary Array, 36-cm	4331247
GeneScan™ 500 LIZ® Size Standard	4322682
OR	*OR*
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Hi-Di™ Formamide	4311320
Running Buffer, 10X	4335613
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
96-Well Plate Septa	4315933
MicroAmp® Optical 96-Well Reaction Plate	N8010560
POP-7™ Polymer for the 3730 Genetic Analyzer	4332241
For a complete list of parts and accessories for the 3730 instrument, refer to Appendix A of the *Applied Biosystems® 3730/3730xl DNA Analyzer Getting Started Guide* (Part no. 4359476).	
PCR Amplification	
MicroAmp® 96-Well Tray	N8010541
MicroAmp® Reaction Tube with Cap, 0.2-mL	N8010540
MicroAmp® 8-Tube Strip, 0.2-mL	N8010580
MicroAmp® 8-Cap Strip	N8010535

Item†	Source
MicroAmp® 96-Well Tray/Retainer Set	403081
MicroAmp® 96-Well Base	N8010531
MicroAmp® Clear Adhesive Film	4306311
MicroAmp® Optical Adhesive Film	4311971
MicroAmp® Optical 96-Well Reaction Plate	N8010560
Other user-supplied materials	
Hi-Di™ Formamide, 25-mL	4311320
Aerosol resistant pipette tips	MLS
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Vortex	MLS

† For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

C Plate Layouts

Example PCR plate layout

The following layout is recommended for use with the sensitivity experiment on page 19. Create 3 identical plates for amplification at 3 different cycle numbers.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Samp 1	Samp 8	Samp 15	Samp 22								
B	Samp 2	Samp 9	Samp 16	Samp 23								
C	Samp 3	Samp 10	Samp 17	Samp 24								
D	Samp 4	Samp 11	Samp 18	Samp 25								
E	Samp 5	Samp 12	Samp 19	Samp 26								
F	Samp 6	Samp 13	Samp 20	Neg ctrl								
G	Samp 7	Samp 14	Samp 21	9947A								
H												

Example electrophoresis plate layout

The following layout is recommended for use with the sensitivity experiment on page 19.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Samp 1	Samp 8	Samp 15	Samp 22	Samp 1	Samp 8	Samp 15	Samp 22	Samp 1	Samp 8	Samp 15	Samp 22
B	Samp 2	Samp 9	Samp 16	Samp 23	Samp 2	Samp 9	Samp 16	Samp 23	Samp 2	Samp 9	Samp 16	Samp 23
C	Samp 3	Samp 10	Samp 17	Samp 24	Samp 3	Samp 10	Samp 17	Samp 24	Samp 3	Samp 10	Samp 17	Samp 24
D	Samp 4	Samp 11	Samp 18	Samp 25	Samp 4	Samp 11	Samp 18	Samp 25	Samp 4	Samp 11	Samp 18	Samp 25
E	Samp 5	Samp 12	Samp 19	Samp 26	Samp 5	Samp 12	Samp 19	Samp 26	Samp 5	Samp 12	Samp 19	Samp 26
F	Samp 6	Samp 13	Samp 20	Neg ctrl	Samp 6	Samp 13	Samp 20	Neg ctrl	Samp 6	Samp 13	Samp 20	Neg ctrl
G	Samp 7	Samp 14	Samp 21	9947A	Samp 7	Samp 14	Samp 21	9947A	Samp 7	Samp 14	Samp 21	9947A
H	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank

Cycle 1 Cycle 2 Cycle 3

D

PCR Work Areas

Work area setup and lab design

Many resources are available for the appropriate design of a PCR laboratory. If you are using the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit for:

- Forensic DNA testing, refer to "Forensic Laboratories: Handbook for Facility Planning, Design, Construction and Moving," National Institute of Justice, 1998
- Parentage DNA testing, refer to the "Guidance for Standards for Parentage Relationship Testing Laboratories," American Association of Blood Banks, 7th edition, 2004

The sensitivity of the Identifiler® Direct Kit (and other PCR-based tests) enables amplification of minute quantities of DNA, necessitating precautions to avoid contamination of samples yet to be amplified (Kwok and Higuchi, 1989).

Also take care while handling and processing samples to prevent contamination by human DNA. Wear gloves at all times and change them frequently. Close sample tubes when not in use. Limit aerosol dispersal by handling sample tubes and reagents carefully.

Note: We do not intend these references for laboratory design to constitute all precautions and care necessary for using PCR technology.

PCR setup work area

IMPORTANT! These items should never leave the PCR Setup Work Area.

- Calculator
- Gloves, disposable
- Marker pen, permanent
- Microcentrifuge
- Microcentrifuge tubes, 1.5-mL, or 2.0-mL, or other appropriate clean tube (for Master Mix preparation)
- Microcentrifuge tube rack
- Pipette tips, sterile, disposable hydrophobic filter-plugged
- Pipettors

- Tube decapper, autoclavable
- Vortex

Amplified DNA work area

IMPORTANT! Place the thermal cyclers in the Amplified DNA Work Area.

You can use the following systems:

- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block

  IMPORTANT! The Identifiler® Direct Kit is not validated for use with the GeneAmp® PCR System 9700 with the Aluminium 96-Well Block. Use of this thermal cycling platform may adversely affect performance of the Identifiler® Direct Kit.

- Veriti® 96-Well Thermal Cycler

E

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Specific chemical handling

CAS	Chemical	Phrase
26628-22-8	Sodium Azide	Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Bibliography

Akane, A., Matsubara, K., Nakamura, H., Takahashi, S., and Kimura, K. 1994. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. *J. Forensic Sci.* 39:362–372.

Bonferroni, C.E. 1936. Teoria statistica delle classi e calcolo Belle probabilita. *Publicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze* 8:3–62.

Barber, M.D. and Parkin, B.H. 1996. Sequence analysis and allelic designation of the two short tandem repeat loci D18S51 and D8S1179. *Intl. J. Legal Med.* 109:62–65.

Baron, H., Fung, S., Aydin, A., Bahrig, S., Luft, F.C., Schuster, H. 1996. Oligonucleotide ligation assay (OLA) for the diagnosis of familial hypercholesterolemia. *Nat. Biotechnol.* 14:1279–1282.

Begovich A.B., McClure G.R., Suraj V.C., Helmuth R.C., Fildes N., Bugawan T.L., Erlich H.A., Klitz W. 1992. Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. *J. Immunol.* 148:249–58.

Bender, K., Farfan, M.J., Schneider, P.M. 2004. Preparation of degraded human DNA under controlled conditions. *Forensic Sci. Int.* 139:134–140.

Brinkman, B., Klintschar, M., Neuhuber, F., Huhne, J. and Rolf, B. 1998. Mutation rate in human microsatellites: Influence of the structure and length of the tandem repeat. *Am. J. Hum. Genet.* 62:1408–1415.

Brinkman, B., Moller, A. and Wiegand, P. 1995. Structure of new mutations in 2 STR systems. *Intl. J. Legal Med.* 107:201–203.

Butler, J.M. 2005. Forensic DNA Typing. Burlington, MA:Elsevier Academic Press.

Butler, J.M., Shen, Y., McCord, B.R. 2003. The development of reduced size STR amplicons as tools for analysis of degraded DNA. J. Forensic Sci. 48:1054–1064.

Chakraborty, R. Kimmel, M., Stivers, D., Davison, L., and Deka, R. 1997. Relative mutation rates at di-, tri-, and tetranucleotide microsatellite loci. *Proc. Natl. Acad. Sci. USA* 94:1041–1046.

Chakraborty, R., Stivers, D., and Zhong, Y. 1996. Estimation of mutation rates from parentage exclusion data: applications to STR and VNTR loci. *Mutat. Res.* 354:41–48.

Chakraborty, R. and Stivers, D.N. 1996. Paternity exclusion by DNA markers: effects of paternal mutations. *J. Forensic Sci.* 41:671–*677.*

Chung, D.T., Drabek, J., Opel, K.L., Butler, J.M. and McCord, B.R. 2004. A study of the effects of degradation and template concentration on the amplification efficiency of the Miniplex primer sets. *J. Forensic Sci*. 49:733–740.

Clark J.M. 1988. Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. *Nucleic Acids Res.* 16:9677–9686.

Coble, M.D. and Butler, J.M. 2005. Characterization of new miniSTR loci to aid analysis of degraded DNA. *J. Forensic Sci.* 50:43–53.

DeFranchis, R., Cross, N.C.P., Foulkes, N.S., and Cox, T.M. 1988. A potent inhibitor of Taq DNA polymerase copurifies with human genomic DNA. *Nucleic Acids Res.* 16:10355.

DNA Advisory Board, Federal Bureau of Investigation, U.S. Department of Justice. 1998. Quality assurance standards for forensic DNA testing laboratories.

Drabek, J., Chung, D.T., Butler, J.M., McCord, B.R. 2004. Concordance study between Miniplex assays and a commercial STR typing kit. *J. Forensic Sci*. 49:859–860.

Edwards, A., Civitello, A., Hammond, H., and Caskey, C. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am. J. Hum. Genet*. 49:746–756.

Edwards, A., Hammond, H.A., Lin, J., Caskey, C.T., and Chakraborty, R. 1992. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics* 12:241–253.

Frank, W., Llewellyn, B., Fish, P., *et al.* 2001. Validation of the AmpFℓSTR® Profiler Plus™ PCR Amplification Kit for use in forensic casework. *J. Forensic Sci.* 46:642–646.

Glock, B., Dauber, E.M., Schwartz, D.W., Mayr W.R. 1997. Additional variability at the D12S391 STR locus in an Austrian population sample: sequencing data and allele distribution. *Forensic Sci. Int.* 90:197–203.

Grossman, P.D., Bloch, W., Brinson, E., Chang, C.C., Eggerding, F.A., Fung, S., Iovannisci, D.M., Woo, S., Winn-Deen, E.S. 1994. High-density multiplex detection of nucleic acid sequences: oligonucleotide ligation assay and sequence-coded separation. *Nucleic Acids Res.* 22:4527–4534.

Grubwieser, P. Muhlmann, R., Berger, B., Niederstatter, H., Palvic, M., Parson, W. 2006. A new "mini-STR-multiplex" displaying reduced amplicon lengths for the analysis of degraded DNA. *Int. J. Legal Med.* 120:115–120.

Guo, S.W. and Thompson, E.A. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361–372.

Guthmiller, J.M., Vargas, K.G., Srikantha, R., Schomberg, L.L., Weistroffer, P.L., McCray, P.B. and Tack, B.F. 2001. Susceptibilities of oral bacteria and yeast to mammalian cathelicidins. *Antimicrob. Agents Chemother.* 45:3216–3219.

Hammond, H., Jin, L., Zhong, Y., Caskey, C., and Chakraborty, R. 1994. Evaluation of 13 short tandem repeat loci for use in personal identification applications. *Am J. Hum. Genet*. 55:175–*189.*

Holt, C., Stauffer, C., Wallin, J., *et al.* 2000. Practical applications of genotypic Surveys for forensic STR testing. *Forensic Sci. Int.* 112:91–109.

Kalinowski, S.T. 2006. HW-QuickCheck: an easy-to-use computer program for checking genotypes for agreement with Hardy-Weinberg expectations. *Molecular Ecology Notes* 6:974–979.

Kimpton, C., Walton, A., and Gill, P. 1992. A further tetranucleotide repeat polymorphism in the vWF gene. *Hum. Mol. Genet.* 1:287.

Kong, X., Murphy, K., Raj, T., He, C., White, P.S., Matise, T.C. 2004. A combined linkage-physical map of the human genome. *Am. J. Hum. Genet.* 75:1143–1148.

Kwok, S., and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237–238.

Lareu, M.V., Pestoni, M.C., Barros, F., Salas, A., Carracedo, A. 1996. Sequence variation of a hypervariable short tandem repeat at the D12S391 locus. *Gene* 182:151–153.

Lazaruk, K., Walsh, P.S., Oaks, F., Gilbert, D., Rosenblum, B.B., Menchen, S., Scheibler, D., Wenz, H.M., Holt, C., Wallin, J. 1998. Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. *Electrophoresis* 19:86–93.

Levene, H. 1949. On a matching problem in genetics. *Ann. Math. Stat.* 20:91–94.

Li, H. Schmidt, L., Wei, M-H., Hustad, T. Leman, M.I., Zbar, B. and Tory, K. 1993. Three tetranucleotide polymorphisms for loci:D3S1352; D3S1358; D3S1359. *Hum. Mol. Genet.* 2:1327.

Magnuson, V.L., Ally, D.S., Nylund, S.J., Karanjawala, Z.E., Rayman, J.B., Knapp, J.I., Lowe, A.L., Ghosh, S., Collins, F.S. 1996. Substrate nucleotide-determined non-templated addition of adenine by Taq DNA polymerase: implications for PCR-based genotyping and cloning. *Biotechniques* 21:700–709.

Mansfield, E.S., Robertson, J.M., Vainer, M., Isenberg, A.R., Frazier, R.R., Ferguson, K., Chow, S., Harris, D.W., Barker, D.L., Gill, P.D., Budowle, B., McCord, B.R. 1998. Analysis of multiplexed short tandem repeat (STR) systems using capillary array electrophoresis. *Electrophoresis* 19:101–107.

Mills, K.A., Even, D., and Murrau, J.C. 1992. Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). *Hum. Mol. Genet*. 1:779.

Momhinweg, E., Luckenbach, C., Fimmers, R., and Ritter, H. 1998. D3S1358: sequence analysis and gene frequency in a German population. *Forensic Sci. Int.* 95:173–178.

Moretti, T., Baumstark, A., Defenbaugh, D., Keys, K., Smerick, J., and Budowle, B. 2001. Validation of short tandem repeats (STRs) for forensic usage: Performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. *J. Forensic Sci.* 46(3):647–660.

Mulero, J.J., Chang, C.W., and Hennessy, L.K. 2006. Characterization of N+3 stutter product in the trinucleotide repeat locus DYS392. *J. Forensic Sci.* 51:826–830.

Nakahori, Y., Takenaka, O., and Nakagome, Y. 1991. A human X-Y homologous region encodes amelogenin. *Genomics* 9:264–269.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA* 70:3321–3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.

Revised Validation Guidelines-Scientific Working Group on DNA Analysis Methods (SWGDAM). *Forensic Science Communications* (July 2004) Volume 6 (3). Available at www.fbi.gov/hq/lab/fsc/current/standards/2004_03_standards02.htm

Sensabaugh, G.F. 1982. Biochemical markers of individuality. In: Saferstein, R., ed. *Forensic Science Handbook*. Prentice-Hall, Inc., New York, pp. 338–415.

Sharma, V. and Litt, M. 1992. Tetranucleotide repeat polymorphism at the D21S11 locus. *Hum Mol. Genet*. 1:67.

Shin, C.H., Jang, P., Hong, K.M,, Paik, M.K. 2004. Allele frequencies of 10 STR loci in Koreans. *Forensic Sci. Int.* 140:133–135.

Smith, R.N. 1995. Accurate size comparison of short tandem repeat alleles amplified by PCR. *Biotechniques* 18:122–128.

Sparkes, R., Kimpton, C., Watson, S., Oldroyd, N., Clayton, T., Barnett, L., Arnold, J., Thompson, C., Hale, R., Chapman, J., Urquhart, A., and Gill, P. 1996a. The validation of a 7-locus multiplex STR test for use in forensic casework. (I). Mixtures, ageing, degradation and species studies. *Int. J. Legal Med.* 109:186–194.

Sparkes, R., Kimpton, C., Gilbard, S., Carne, P., Andersen, J., Oldroyd, N., Thomas, D., Urquhart, A., and Gill, P. 1996b. The validation of a 7-locus multiplex STR test for use in forensic casework. (II), Artifacts, casework studies and success rates. *Int. J. Legal Med*. 109:195–204.

Straub, R.E., Speer, M.C., Luo, Y., Rojas, K., Overhauser, J., Ott, J., and Gilliam, T.C. 1993. A microsatellite genetic linkage map of human chromosome 18. *Genomics* 15:48–56.

Suido, H., Nakamura, M., Mashimo, P.A., Zambon, J.J., and Genco, R.J. 1986. Arylaminopeptidase activities of the oral bacteria. *J. Dent. Res.* 65:1335–1340.

Waiyawuth, W., Zhang, L., Rittner, C., Schneider, P.M. 1998. Genetic analysis of the short tandem repeat system D12S391 in the German and three Asian populations. *Forensic Sci. Int.* 94:25–31.

Wallin, J.M., Buoncristiani, M.R., Lazaruk, K.D., Fildes, N., Holt, C.L., Walsh, P.S. 1998. SWGDAM validation of the AmpFlSTR blue PCR amplification kit for forensic casework analysis. *J. Forensic Sci.* 43:854–870.

Wallin, J.M., Holt, C.L., Lazaruk, K.D., Nguyen, T.H., Walsh, P.S. 2002. Constructing universal multiplex PCR systems for comparative genotyping. *J. Forensic Sci*. 47:52–65.

Walsh, P.S., Fildes, N.J., Reynolds, R. 1996. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Res.* 24:2807–2812.

Weber, J. and Wong, C. 1993. Mutation of human short tandem repeats. *Hum. Mol. Genet*. 2:1123–1128.

Weir, B. 1990. Genetic Data Analysis. *Sinauer Associates* Sunderland, MA

Wiegand, P. and Kleiber, M. 2001. Less is more—length reduction of STR amplicons using redesigned primers. *Int. J. Legal Med*. 114:285–287.

Documentation and Support

Related documentation

Document title	Part number
3100/3100-Avant Data Collection v2.0 User Guide	4347102
3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin	4350218
3100 Genetic Analyzer User Manual (Data Collection v1.1)	4315834
3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin	4332345
Applied Biosystems® 3130/3100xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin	4363787
Applied Biosystems® 3130/3130xl Genetic Analyzers Getting Started Guide	4352715
Applied Biosystems® 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide	4352716
Applied Biosystems® 3130/3130xl Genetic Analyzers Quick Reference Card	4362825
Applied Biosystems® 3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide	4359472
Applied Biosystems® 3130/3100xl DNA Analyzers User Guide	4331468
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Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide, Data Collection v1.0	4401661
Applied Biosystems® 3500/3500xL Genetic Analyzer User Bulletin: Solutions to issues related to software, data, hardware, and consumables **Note:** Additional user bulletins may be available at **www.lifetechnologies.com**	4445098
Applied Biosystems® 3730/3730xl Genetic Analyzer Getting Started Guide	4359476
GeneAmp® PCR System 9700 Base Module User's Manual	N805-0200
Quantifiler® Kits: Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual	4344790
AmpFℓSTR® Identifiler® PCR Amplification Kit User's Manual	4323291
GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide	4338775
GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial	4335523
Installation Procedures and New Features for GeneMapper® ID Software v3.2 User Bulletin	4352543
GeneMapper® ID-X Software Version 1.0 Getting Started Guide	4375574
GeneMapper® ID-X Software Version 1.0 Quick Reference Guide	4375670
GeneMapper® ID-X Software Version 1.0 Reference Guide	4375671
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Getting Started Guide	4396773
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Quick Reference Guide	4402094
GeneMapper® ID-X Software Version 1.2 Reference Guide	4426481

Document title	Part number
GeneMapper® ID-X Software Version 1.2 Quick Reference Guide	4426482

Portable document format (PDF) versions of this guide and the documents listed above are available at **www.lifetechnologies.com**.

Note: To open the user documentation available from the our web site, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**.

Obtain SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtain support

For HID support:

- **In North America** – Send an email to **HIDTechSupport@lifetech.com**, or call **888-821-4443 option 1**.
- **Outside North America** – Contact your local support office.

For the latest services and support information for all locations, go to:

www.lifetechnologies.com

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- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
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- Obtain information about customer training
- Download software updates and patches

Notices

Limited Product Warranty

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